

1973

# Physio-pathologic studies in induced parturition in the cow (*Bos taurus*)

Lawrence Eugene Evans  
*Iowa State University*

Follow this and additional works at: <https://lib.dr.iastate.edu/rtd>



Part of the [Animal Sciences Commons](#), and the [Veterinary Medicine Commons](#)

---

## Recommended Citation

Evans, Lawrence Eugene, "Physio-pathologic studies in induced parturition in the cow (*Bos taurus*) " (1973). *Retrospective Theses and Dissertations*. 5080.

<https://lib.dr.iastate.edu/rtd/5080>

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact [digirep@iastate.edu](mailto:digirep@iastate.edu).

## INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.
2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.
3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.
4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.
5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

**Xerox University Microfilms**

300 North Zeeb Road  
Ann Arbor, Michigan 48106

74-9113

EVANS, D.V.M., Lawrence Eugene, 1938-  
PHYSIO-PATHOLOGIC STUDIES IN INDUCED  
PARTURITION IN THE COW (BOS TAURUS).

Iowa State University, Ph.D., 1973  
Veterinary Science

University Microfilms, A XEROX Company, Ann Arbor, Michigan

Physio-pathologic studies in induced  
parturition in the cow (Bos taurus)

by

Lawrence Eugene Evans

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
The Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

Major: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University  
Ames, Iowa

1973

## TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. REVIEW OF THE LITERATURE	3
A. Fetal Autonomy	3
B. Maternal Steroid Levels in Pregnancy and Parturition	14
1. Progesterone	14
2. Estrogens	25
3. Corticoids	30
C. Induction Studies in Domestic Animals	32
D. Control of Parturition	36
E. Postpartum Reproductive Function	53
1. Postpartum fertility	53
2. Follicular growth	54
3. First estrus and first ovulation	55
4. Uterine involution	58
5. Exogenous hormone treatments	65
III. MATERIALS AND METHODS	68
A. Experimental Design and Animals	68
1. Experiment I	68
2. Experiment II	69
3. Experiment III	73
B. Steroid Assays	74
1. General procedures	74
2. Corticosteroids	75
3. Progesterone	75
4. Estrogens	76
C. Statistical Analysis	77

	Page
IV. RESULTS	80
A. Postpartum Reproductive Involution	80
1. Gross observations	80
2. Histological observations in normal cows	83
3. Histological observations in DXMS treated cows	90
4. Histological comparison of normal and DXMS treated cows	92
B. Steroid Levels in DXMS Treated Cows	106
1. Corticosteroids	106
2. Progesterone	114
3. Estrogens	120
4. E:P ratio	128
C. Effects of HCG in DXMS Treated Cows	128
V. DISCUSSION	141
A. Postpartum Reproductive Function	141
B. Relationship of DXMS Treatment and Steroid Changes at Parturition	152
VI. CONCLUSION	169
VII. LITERATURE CITED	173
VIII. ACKNOWLEDGEMENTS	195
IX. APPENDIX	196

## LIST OF FIGURES

	Page
Figure 1. Photomicrograph of caruncle from seven day postpartum cow	94
Figure 2. Photomicrograph of cryptal giant cells	94
Figure 3. Photomicrograph of caruncular arteriole from seven day postpartum cow	94
Figure 4. Photomicrograph of intercaruncular endometrium from seven day postpartum cow	94
Figure 5. Photomicrograph of caruncle from 14 day postpartum cow	96
Figure 6. Photomicrograph of septal artery from 14 day postpartum cow	96
Figure 7. Photomicrograph of intercaruncular endometrium from 14 day postpartum cow	96
Figure 8. Photomicrograph of caruncle from 28 day postpartum cow	96
Figure 9. Photomicrograph of septal artery from 28 day postpartum cow	98
Figure 10. Photomicrograph of intercaruncular endometrium from 28 day postpartum cow	98
Figure 11. Photomicrograph of caruncle from 42 day postpartum cow	98
Figure 12. Photomicrograph of corpus luteum of pregnancy from seven day postpartum cow	98
Figure 13. Photomicrograph of corpus luteum of pregnancy from 14 day postpartum cow	100
Figure 14. Photomicrograph of Call-Exner bodies	100
Figure 15. Photomicrograph of corpus luteum of pregnancy from 28 day postpartum cow	100
Figure 16. Photomicrograph of intercaruncular endometrium from 14 day postpartum DXMS treated cow	102

Figure 17.	Photomicrograph of myometrium from 14 day postpartum DXMS treated cow	102
Figure 18.	Photomicrograph of intercaruncular endometrium from 28 day postpartum DXMS treated cow	102
Figure 19.	Graphic illustration of histological evaluation of uterine involution in DXMS treated cows	105
Figure 20.	Jugular plasma corticoids in DXMS treated cows before and after parturition	108
Figure 21.	Jugular plasma corticoids in naturally calving cow 20	110
Figure 22.	Plasma corticoids in jugular, uterine and utero-ovarian veins of DXMS treated cows	112
Figure 23.	Jugular plasma progesterone in DXMS treated cows calving, not calving and in naturally calving cow	116
Figure 24.	Plasma progesterone in jugular, utero-ovarian and uterine veins of DXMS treated cows	118
Figure 25.	Jugular plasma estradiol in DXMS treated cows	122
Figure 26.	Jugular plasma estrone in DXMS treated cows calving, not calving and in naturally calving cow	124
Figure 27.	Plasma estrone in jugular and utero-ovarian veins of DXMS treated cows before and after parturition	126
Figure 28.	Jugular plasma estrogens and plasma progesterone in DXMS treated cows before and after parturition	130
Figure 29.	Jugular plasma estrogens:progesterone ratio in DXMS treated cows at parturition and in a naturally calving cow	132



Figure 30.	Jugular plasma progesterone in HCG and DXMS treated cows	135
Figure 31.	Jugular plasma estrone in HCG and DXMS treated cows	137

## LIST OF TABLES

	Page
Table 1. Post mortem evaluation of reproductive involution	81
Table 2. Ante mortem evaluation of reproductive involution	82
Table 3. Plasma corticoids in DXMS treated cows	113
Table 4. Plasma progesterone in DXMS treated cows	119
Table 5. Plasma estrone in DXMS treated cows	127
Table 6. Plasma progesterone in HCG treated cows	138
Table 7. Plasma estrone in HCG treated cows	139
Table 8. Plasma progesterone in HCG treated cows	139
Table 9. Plasma estrone in HCG treated cows	140
Appendix Table 1. Corticoid assay procedure	197
Appendix Table 2. Progesterone assay procedure	200
Appendix Table 3. Estrogen assay procedure	205
Appendix Table 4. Preparation of rabbit cytosol solution	210

## I. INTRODUCTION

Recently researchers have found that glucocorticoids, when administered at sufficient dosages during the later stages of gestation, can initiate parturition in the ruminant. If the drug is administered after 270 days in cattle or 140 days in sheep, viable offspring can be expected within 36-72 hours. Earlier treatments, usually requiring larger dosages, often result in abortion or premature offspring.

This procedure appears to have a variety of uses as a management tool in the cattle industry. It allows for grouping of working hours so that calving can be observed and assisted where indicated. Cattle with a predictable high incidence of dystocia, such as primipara or those bred to produce heavy calves can be delivered earlier. In addition, assistance in the delivery of the calf reduces the incidence of stillbirth. It is a most appealing procedure in disease problems of late pregnancy. Prolonged pregnancies, severe udder edema, fractures, ruptured prepubic tendon, and hydrops allantois or amnion are examples of conditions where induction of parturition may prove to be advantageous.

Within 24 hours post treatment, cows show relaxation of the perineal region, vulvar edema and udder filling. Loss of the cervical seal occurs shortly thereafter. Parturition is usually fast and uncomplicated, although vaginal relaxation and dilation is less complete when compared to a normal

parturition.

A high percentage of induced cows have retained placentae. The incidence increases as the gestational period is shortened. Traditionally, retained placentae have been associated with endometritis, metritis or pyometritis. Poor uterine involution and delayed fertility are implicated as part of this problem.

This study was undertaken, not to prove the effectiveness of glucocorticoids in inducing parturition, but to elucidate some of the physio-pathological changes during induced parturition and the subsequent postpartum period.

## II. REVIEW OF THE LITERATURE

### A. Fetal Autonomy

Parturition is the climax of pregnancy, the finale in the cycle of reproduction. For all its apparent simplicity and wonder, a complete understanding of the physiological changes associated with the sudden advent of labor and the termination of pregnancy still eludes the researcher.

There is growing evidence that the fetus participates in the initiation of labor, though fetal endocrine function may not be vital to its occurrence. Removal of the fetuses but not the placenta in rats and mice results in the placenta being delivered at term (Marshall and Moir, 1952). Fetal death in the human results in labor and delivery about one week later. However, in the normal fetus, certain endocrine functions are altered near term and appear to influence the duration of pregnancy.

It is also evident that fetal genotype figures prominently in pregnancy duration. This is best exemplified by the altered duration of pregnancy in the equine species with crossbred foals such as the mule or hinny. With these foals the duration of gestation is approximately intermediate between the duration of straight bred foals. In cattle, females are generally born earlier than males which are bigger and heavier (Roberts, 1971).

Conditions of prolonged pregnancy in cattle have demonstrated that portions of this genetic control are dependent upon a fetal endocrine mechanism. Kennedy, Kendrick, and Stormont (1957) described prolonged gestation in the Guernsey breed. The calves were born post-term with development approximating a seven month fetus. Adenohypophyseal aplasia was also detected. Holm, Parker, and Galligan (1961) described a condition of prolonged gestation in Holstein-Friesian calves, which also involved dysfunction of the adenohypophyseal system. These calves were unusually large at delivery, but the adenohypophysis was smaller than normal. The adrenal glands were significantly smaller than those of normal calves. Delivery by Cesarean section resulted in signs of adrenal insufficiency and death within a few hours unless adrenocorticoid therapy was initiated. Holm, Parker and Galligan (1961) linked this defect to a single recessive autosomal gene.

Rea (1898) associated fetal anencephaly with prolonged pregnancy in women. More recent reviews demonstrate considerable variance among human fetuses due to the variable nature of the malformations and associated hydramnios (Comerford, 1965; Beischer, Brown, and Townsend, 1969). Hydramnios shortened gestation presumably by its volumetric effect on the uterus. In the absence of hydramnios prolonged pregnancy was accompanied by variable development of the adenohypophysis and

always by adrenal hypoplasia. Comerford (1965) reported that patients with an anencephalic fetus do not readily respond to oxytocin even when hydramnios is absent. In many cases urinary excretions of estriol during such gestations is exceedingly low presumably due to a lack of steroid precursors from the fetal adrenal (Beischer, Brown and Townsend, 1969).

There is substantial evidence in sheep that an intact fetal adenohypophysis is essential for normal gestation periods and parturition. Binns et al. (1959) associated unduly prolonged gestation periods with congenital malformation of the head in lambs. Defects ranging from arhinencephaly to cyclops formation with disorganization of the hypothalamus and pituitary gland resulted in prolonged pregnancies of several days to months. This condition is caused by a teratogen in skunk cabbage (Veratrum californicum) when ingested at 14 days after conception (Binns et al., 1963).

A similar malady in South West Africa involving the Karakul pelt industry was reviewed by Van Rensburg (1971). Post-mature fetuses with worthless overgrown pelts were born a few days to months late. Progressive hypophyseal and adrenal atrophy following ingestion of the shrub, Salsola tuberculata, during the last 50 days of gestation suggests a fetal hypothalamic inhibitor.

The converse of the above entities is shortened gestations of a non-infectious nature which are associated with altered

activity of the fetal hypothalamic-pituitary adrenal axis. This is best exemplified with the habitually aborting Angora goats described by Van Rensburg (1971). Abortion in the Angora goat appears to be related to an abnormally low level of adrenal function in the doe and possibly some qualitative changes in adrenal steroid biosynthesis. The situation is associated with a high priority for hair growth induced by intensive selection and inbreeding. Gestational failure is most prevalent in the heavier older goats which were found to have enlarged pituitaries and adrenal hyperplasia as an adaptive response to pregnancy. Those that could maintain adequate adrenal function maintained their pregnancies, while sudden adrenal atrophy in the doe occurred just prior to abortion. Abortions were most frequent near 100 days of gestation, a time of rapid fetal growth. The aborted kids consistently had adrenal hyperplasia. Van Rensburg theorized that fetal stress, probably due to poor placental support by the doe stimulates the fetal hypothalamic-pituitary-adrenal axis and initiates parturition.

Liggins, Kennedy and Holm (1967) demonstrated the experimental counterpart of this phenomenon. Fetal pituitaries were electrocoagulated in 23 ewes between 93 and 143 days gestation. When 70% or more of the fetal pituitary was destroyed before day 134, pregnancy continued until interrupted by Cesarean section 10-30 days beyond term. Many lambs were alive at Cesarean delivery, but all suffered from marked



hypoplasia of fetal adrenal cortex. In multiple pregnancies spontaneous parturition occurred at term unless the pituitaries of all the fetuses were destroyed. These observations suggest participation of the fetal pituitary in parturition through the adrenal cortex of the fetus. Corticotrophin (ACTH) was infused into one of twin lambs when the pregnancy had continued 10 days beyond term. Spontaneous delivery occurred after six days of continuous infusion. The adrenals of the ACTH infused lamb weighed 740 mg while the adrenals of its twin weighed 293 mg.

Comline, Silver and Silver (1970a) produced similar results of prolonged pregnancy and fetal adrenal hypoplasia with fetal hypophysectomy. Complete adrenalectomy of sheep fetuses resulted in failure of the ewes to undergo parturition (Drost and Holm, 1968). Pregnancy lasted 157 days or more. Twin pregnancies were delivered at term if one fetus was left intact.

Liggins (1968) further demonstrated the essential role of the fetal lamb in the mechanism of initiating parturition through the hypothalamic-pituitary-adrenal system. Continuous administration of corticotrophin (ACTH) to eight single fetal lambs at gestational ages 88-129 resulted in spontaneous delivery after four to seven days. Dosages of ACTH ranged from 0.1 mg to 1.0 mg per day. Adrenal weights at birth were nearly equal to the normal term fetus ( $680 \pm 158$  mg). Twin pregnancies did not terminate following infusion of ACTH to only one fetus. Infusions of cortisol into the fetus at a rate of 50 mg/24

hours caused two single lambs to be delivered in 30 to 36 hours, although 25 mg per day had no effect on two single fetuses. Neither cortisol at 100 mg per 24 hours for 72 hours nor prolonged administration of ACTH to the ewe caused parturition.

There is ample evidence of fetal adrenal function throughout most of the duration of pregnancy particularly during the later stages leading to parturition. Comline and Silver (1961) observed that the fetal adrenals of sheep double in size between days 135 and parturition although the medulla remains nearly constant in size during this period. One function of the fetal adrenal is corticosteroid production, cortisol being the most abundant corticosteroid found in the fetal sheep adrenal (ChesterJones et al., 1964; Alexander et al., 1968).

Plasma concentration of corticosteroids in fetal lambs increase several-fold during the last three or four days (Alexander et al., 1968; Bassett and Thorburn, 1969; Comline et al., 1970b; Dixon et al., 1970; Anderson et al., 1972a; Thompson, 1973). Such increased levels were independent of the maternal levels, thus indicative of low placenta transfer (Bassett and Thorburn, 1969 and Dixon et al., 1970). Fetal plasma corticosteroids averaged 2 µg/100 ml until day 130. Highest levels were reached at the time of birth when they averaged 12-15 µg/100 ml plasma (Bassett and Thorburn, 1969). Paisey and Nathanielsz (1971) found a sharp drop in plasma

corticosteroids in the lamb by 3 days postpartum.

Anderson et al. (1972a) studied the in vitro metabolism of isotopically labelled pregnenolone and progesterone by adrenal tissue from fetal and newborn sheep. Fetal lambs ranged in age from 116 days of gestation to newborn and some were stimulated with synacthen (ACTH) prior to removal of the adrenals. All fetal adrenals extensively metabolized the pregnenolone, and after 133 days or more, a large portion was converted to corticosteroids. Adrenal tissue at 116 days and 122 days yielded more corticosteroids than the non-stimulated glands of equal age. The authors attributed this to activation of the 11  $\beta$ -hydroxylase enzyme. Thus 11-deoxycortisol and 11-deoxycorticosterone were converted to cortisol and corticosterone respectively. In in vitro studies radioactive labelled dehydroepiandrosterone was metabolized primarily to androstenedione by the 116 day fetal adrenal but more 11  $\beta$ -hydroxyandrostenedione was present in the 143 day fetal adrenal. This indicated 11  $\beta$ -hydroxylase activity in converting androstenedione to 11  $\beta$ -hydroxyandrostenedione was highest in adrenals of late term lambs. It appears that 11  $\beta$ -hydroxylase enzyme activity increases in near term fetal lambs whether it is a natural or ACTH induced parturition.

The efficiency of 11-deoxycortisol was compared to cortisol as an inducer of premature parturition in the lamb by Anderson et al. (1972a). Cortisol at a dose of 50 mg/day infused into the 113 to 116 day fetal lamb caused parturition

in about 70 hours. In contrast, 11-deoxycortisol, a cortisol precursor, had no effect on parturition when used in a similar manner for as long as eight days.

Nathanielsz et al. (1972) demonstrated that the fetal corticoid response is greater and faster in the near term lamb. E-pyrogen stimulated a two to three fold increase in plasma cortisol when infused into the near term fetus. Lambs stimulated as late as 11 to 14 days before parturition showed only a minimal rise in plasma cortisol.

The fetal pituitary adrenal axis has been investigated to a lesser extent in other species. Jackson and Piasecki (1969) found an adrenal secretion equivalent to 0.3 mg/kg/day in the canine fetus about one week before delivery. There was also good evidence of an increased response to exogenous ACTH in late gestation.

Balfour (1953) determined that cortisol was the major secretory product in newborn calves and these levels fell rapidly the first four days of age. Corticosterone did not appear until 10 days of age. Mature cattle reach a ratio of nearly 1:1 cortisol to corticosterone.

Fetal goat plasma corticosteroids were found to rise sharply before ACTH (Synacthen) induced parturition. Levels of 15 to 16 µg/100 ml were reached at the time of parturition (Thorburn et al., 1972). They observed that ACTH administered to one of twin goat fetuses did not induce premature

parturition. Corticosteroid levels were elevated in the infused fetus, but were not significantly altered in the untreated fetus.

While there is good evidence that the fetal adrenal is functional in initiating parturition, at least in ruminants, this relationship is less clear in humans. Smith and Shearman (1972) did not observe any relationship between human fetal corticosteroid levels at 35 to 37 weeks of gestation occurring concurrently with a rapid increase in hepatic glycogen and amniotic fluid lecithin. Arterial mean levels were less than venous levels at each gestational age, suggesting the fetus is not a contributor of corticoids.

Murphy (1973) reported that cortisol levels in the human fetus change during pregnancy in a manner similar to the corticoid levels of fetal sheep. During early gestation cortisol levels in the human fetus were very low, about 7 ng/ml, but had risen to 23 ng/ml by late pregnancy (36 to 41 weeks). Cortisol levels were considerably elevated (72 to 87 ng/ml) during spontaneous delivery, either vaginal or by Cesarean section. In contrast, induced labor did not alter fetal cortisol levels appreciably. Fetal cortisone levels also rose during pregnancy from 31 ng/ml at 12 to 18 weeks to 87 ng/ml in late gestation. A further rise to about 175 ng/ml occurred with spontaneous and induced labor. Murphy suggested that fetal cortisol levels reflected autonomous fetal adrenal

function, while fetal cortisone levels were proportional to maternal cortisol crossing the placenta and in the process being converted to cortisone. Low levels of fetal cortisol in babies born after induced labor suggest that higher levels with spontaneous labor are not merely secondary to labor.

A second major type of steroid provided by the fetal adrenals serves as precursor for placental synthesis of estrogen. These precursors are androgens, mainly 16  $\alpha$ -hydroxy-dehydroepiandrosterone sulfate and dehydroepiandrosterone sulfate in the human (Siiteri and MacDonald, 1966; Solomon, 1966) while androstenedione is the primary precursor of animals (Ryan, 1969).

The placenta synthesizes little if any steroid de novo but actively converts maternal and fetal 3  $\beta$ -hydroysteroid precursors into the corresponding  $\alpha$ ,  $\beta$ -unsaturated ketones (Diczfalusy, 1964 and Davies, Ryan and Petro, 1970). Four basic enzyme systems are active in placental steroid synthesis; Sulfatases, 3  $\beta$ -hydroxysteroid dehydrogenase, aromatizing enzyme and other dehydrogenases.

Progesterone and 17  $\alpha$ -hydroxyprogesterone from the placenta are secreted to both the maternal and fetal organism. As a result of placental progesterones reaching the fetal adrenal, the fetus is capable of synthesizing most of the adrenocortical hormones and androgens, (C-19 steroids) the bulk of which are converted into estrogens by the placenta.

These estrogens are primarily estrone or estradiol in animals (Davies, Ryan and Petro, 1970) and also substantial quantities of estriol in pregnant women (Tulchinsky et al., 1972).

Following secretion into the maternal compartments, estrone and/or estradiol are converted to a large number of metabolites including estriol, which are then conjugated with glucuronide or sulfate units before excretion in the urine (Siiteri and MacDonald, 1966). The importance of the human fetus in estrogen production is exemplified in hydatiform mole pregnancies, in anencephalic fetuses or following fetal death. Estriol is noticeably lower in these cases (Siiteri and MacDonald, 1966; Beischer, Brown and Townsend, 1969; Tulchinsky, Hobel and Korenman, 1971).

Kim et al. (1971) reported the rather unique observation of steroid synthesis with an ovarian pregnancy. Plasma levels of HCG, progesterone, estradiol-17  $\beta$  and estrone were followed for 16 weeks after the living fetus was removed but the placenta left in situ. While HCG and progesterone levels remained virtually unchanged, both estrone and estradiol levels dropped dramatically after fetal delivery.

The in vitro conversion of  $^{14}\text{C}$  labelled dehydroepiandrosterone and androstenedione to estrogens by placental tissues from the sheep, cow, horse and pig was demonstrated by Ainsworth and Ryan (1966). There was a lack of detectable estrogen synthesis following incubation of placental

preparations with pregnenolone or progesterone substrates. Ainsworth, Deaner and Ryan (1969) reported a similar finding with in vitro studies using placental tissues of monkeys and baboons.

Davies, Ryan and Petro (1970) established that both adrenal tissue (fetal or maternal) and the placenta are needed for conversion of pregnenolone to estrone and estradiol in the sheep and monkey.

As with human placental studies, the estrogens produced by the fetal placenta of sheep and cow are mostly estrone and estradiol sulphoconjugates (Findlay and Seamark, 1971; Pierrepont et al., 1971). The maternal adrenal is also an important source of C-19 steroids as precursors for placental estrogen synthesis in women (Siiteri and MacDonald, 1966) and sheep (Thompson, 1973).

## B. Maternal Steroid Levels in Pregnancy and Parturition

### 1. Progesterone

A comparison of hormone levels in different species before and during parturition emphasized the complexity of endocrine regulation of parturition in different species. Both progesterone and estrogens are present in substantial amounts during pregnancy in most mammals. Their relative and absolute amounts appear to be important in pregnancy maintenance.



Ryan (1971), in his review of "Endocrine Control of Gestational Length", categorized several species in groups based on short or long gestational periods, their dependence upon ovarian hormones for pregnancy and their placental steroid production. The rabbit, rat and dog are short term gestation animals which usually abort after oophorectomy. Oophorectomy in the rat at day 17 or less caused an abrupt drop in plasma progesterone from about 110 ng/ml to 9.0 ng/ml and abortion in 48 hours (Csapo and Wiest, 1969). Exogenous progesterone prevented the results of ovariectomy. Plasma progesterone concentration in intact rats increased to about 110 ng/ml at 14 to 20 days and fell sharply after day 20 of pregnancy to 10 to 20 ng/ml before parturition (Gota and Eik-Nes, 1967; Wiest, 1970). As the plasma progesterone level fell, 20  $\alpha$ -dihydroprogesterone, a progesterone metabolite synthesized in corpora lutea, increased. However, 10 ng/day of 20  $\alpha$ -dihydroprogesterone failed, in itself, to affect parturition (Wiest, 1970). The guinea pig is also a short gestation species, but oophorectomy after mid-term does not result in abortion. This is indicative of an extraovarian source of progesterone.

Heap and Deanesly (1966) showed that ovariectomy did not significantly alter plasma progesterone levels in the guinea pig during the latter half of pregnancy nor did it cause abortion. Plasma progesterone levels are exceedingly high in the pregnant guinea pig even at the time of parturition. Heap and Deanesly (1966) reported progesterone levels of 64 ng/ml

at 11 to 16 days with a peak level of 266 ng/ml at 30 to 45 days. Plasma progesterone levels fell gradually to 140 ng/ml immediately before parturition. Though levels varied slightly, Challis, Heap and Illingworth (1971b) reported much the same fluctuation in progesterone levels in the pregnant guinea pig. An increase of such magnitude was partially the result of a sharp decrease in the mean clearance rate of progesterone after 15 days (Illingworth, Heap and Perry, 1970) and an increased level of plasma transcortin, a plasma protein with a high affinity for progesterone (Seal and Doe, 1966). Protein binding of progesterone protects the steroid from rapid metabolism by the liver and extra hepatic tissues.

Ryan (1971) listed two domestic animals, the sow and goat, as long gestation period animals, that are dependent upon the ovary and its corpora lutea for maintenance of pregnancy. In the goat, plasma progesterone remains steady during early pregnancy averaging 2.5 to 3.5 ng/ml up to day 60 and increasing to 4.5-5.5 ng/ml until one to two days before parturition. Maternal plasma progesterone remained at 1.0 to 1.5 mg/ml for 36 to 48 hours prior to parturition in the goat (Thorburn and Schneider, 1971; Thorburn et al., 1972). Blom and Lyngset (1971) observed slightly higher levels, but also recorded a profound drop in maternal progesterone one to two days before parturition.

Ovariectomy resulted in depleted plasma progesterone levels within five to six hours (Thorburn and Schneider, 1971) and a significant drop in progesterone as early as 10 minutes if both the ovary and uterus were removed (Blom and Lyngset, 1971).

Linzell and Heap (1968) and Blom and Lyngset (1971) observed that the gravid horn of the uterus removed significant quantities of progesterone. Mean arterio-venous differences for the uterus average 4-7 ng/ml plasma at 120 days but this decreased to about 1.0 ng/ml near term.

Killian, Garnerick and Day (1972) reported jugular plasma progesterone levels averaging 6 ng/ml through the day of parturition in sows, but decreased to an average of 1-2 ng/ml by 24 hours post-partum where it remained for the next six days. Molokwu and Wagner (1973) observed similar levels of progesterone prior to parturition, but also noted plasma progesterone levels fell abruptly two to three days before parturition. This is consistent with the findings of Kimura and Cornwell (1938) who reported a precipitous drop in corpora lutea content of progestin just prior to parturition.

The mare, ewe, cow and woman all have long gestational periods. In addition each can maintain pregnancy for extended periods after oophorectomy in the latter half of pregnancy (Ryan, 1971).

In the mare Short (1960) found the progesterone level remained high in the fetal cord blood at parturition. Peripheral plasma progesterone in the mare was consistently low after five months of pregnancy.

Plasma progesterone in pregnant women is primarily of placental origin except for a short period of corpus luteum dependence (Fuchs, Fuchs and Short, 1963). Yannone, McCurdy and Goldfien (1968) and Llauro, Runnebaum and Zander (1968) found increased concentrations of progesterone with advancing pregnancy. Progesterone levels averaged 140 to 190 ng/ml and showed no significant decrease until labor had commenced. Within 24 hours after placental passage plasma progesterone levels were down to 19-40 ng/ml. Women with a multiple pregnancy had higher plasma progesterone levels than a single pregnancy (Short, 1961), Csapo et al. (1971b) found a similar level of plasma progesterone in 12 pregnant women. Five patients with low values were in labor for an average of 2.8 hours while six patients with higher progesterone levels (average 169 ng/ml) were in active labor an average of eight hours. There are conflicting reports concerning plasma progesterone in induced abortion. Wiest et al. (1970) and Holmdahl, Johansson and Nilsson (1971) reported a significant decrease in maternal plasma progesterone after an intra-amniotic injection of hypertonic saline at 13 to 20 weeks of pregnancy. However, Holmdahl, Johansson and Nilsson (1971) found that these decreased levels did not correlate well with

commencement of labor.

Short et al. (1965) reported that intra-amniotic injection of hypertonic saline during midpregnancy had little effect on progesterone concentrations in the uterine venous blood.

Prostaglandin ( $\text{PGF}_{2\alpha}$ ) infused intravenously as an abortifacient during midgestation was found to reduce plasma progesterone by 50% prior to abortion (Csapo, Sauvage and Wiest, 1971a; Lehmann et al., 1972). Progesterone levels in non-aborting women remained at pre-infusion levels (Csapo, Sauvage and Wiest, 1971a).

Speroff et al. (1972) did not find a significant drop in either plasma progesterone or estriol after  $\text{PGF}_{2\alpha}$  infusion during mid-pregnancy. Neither infusion of oxytocin or  $\text{PGF}_{2\alpha}$  altered the plasma levels of either progesterone or estriol in term pregnant women (LeMaire et al., 1972)

In the ewe, the peripheral concentration of progesterone reaches its highest value during the last two to three weeks of gestation and falls before the time of delivery (Bassett et al., 1969; Fylling, 1971; Challis, Harrison and Heap, 1971a; Bedford et al., 1972; Stabenfeldt, Drost and Franti, 1972; Thompson, 1973).

Progesterone levels increased gradually from 2-3 ng/ml to a peak at 120-140 days of 12-14 ng/ml for single pregnancies, and about 20 ng/ml for twin pregnancies. There is a marked but late drop in progesterone levels to 1-2 ng within the last 24

hours of parturition. Short and Moore (1959) failed to detect any significant drop in progesterone levels prior to parturition but found a rapid fall after birth. Their highest levels were 4.1 ng/ml with little difference noted between single and twin pregnancies.

The difference in progesterone levels reported by Short and Moore (1959) and the other before mentioned researchers was basically one of refined technique. Short and Moore used U.V. spectrophotometry while most of the others utilized a competitive protein binding technique.

It is suggested that the majority of progesterone in the pregnant ewe is of placental origin after 50 days. Short and Moore (1959) found that placental concentrations of progesterone increased in the ovariectomized pregnant ewe. This suggests a compensating response following ovariectomy. Hemispayed pregnant ewes lacking an ovary containing a corpus luteum had higher progesterone levels in the utero-ovarian venous plasma than similar ewes which retained their corpora lutea during the last two months of pregnancy (Mattner and Thorburn, 1971).

Bedford et al. (1972) and Thompson (1973) found a significant difference between uterine and jugular vein levels of progesterone. Uterine samples averaged approximately five times the peripheral levels. Linzell and Heap (1968) reported a similar difference in progesterone levels between the uterine and ovarian vein. While it seems apparent that the corpora

lutea do influence placental progesterone, the majority of maternal plasma progesterone is of placental origin in the ewe.

The cow appears to be intermediate between those animals such as the sow, goat, rat and rabbit, in which the corpus luteum is necessary for maintenance of pregnancy and those species such as the ewe, mare, and women which require little or no ovarian support for maintenance of pregnancy in the last half of pregnancy.

Removal of the corpus luteum during early pregnancy causes immediate abortion in the cow. However, the corpus luteum of the cow may be removed during late pregnancy without causing immediate abortion. McDonald, McNutt and Nichols (1953) removed the corpus luteum in five cows between days 207 and 236. Three of these cows carried live fetuses for 18, 24, and 50 days until progesterone therapy was initiated. Estergreen et al. (1967) found ovariectomy on days 139 to 174 resulted in abortion at an average of 11 days later in seven of eight cows. Ovariectomy performed during the periods of 200-210, 226-237 and 251-258 resulted in abortion at an average gestation length of 256, 264, and 265 days respectively. Subnormal cervical dilation prior to parturition and calving difficulties were common in these cows. As a result of early parturition, weak calves and retention of the fetal placental membranes were common in ovariectomized cows.

Erb et al. (1968) demonstrated that ovarian venous plasma was low in progesterone from days 199 to 237 in the intact cow but increased in concentration during the last month of pregnancy to exceed by two-threefold the peripheral plasma levels. Stormshak and Erb (1961) had previously demonstrated that the corpus luteum (CL) after regressing slightly, remained fairly constant in size from four to five months to term. Progesterone content of the CL was found to increase to a level of 18.7  $\mu\text{g/gm}$  in the last trimesters of pregnancy (Stormshak and Erb, 1961).

Mills and Morrisette (1970) demonstrated, with in vitro perfusion studies, that the bovine ovary of late pregnancy was able to synthesize progesterone at a rate similar to bovine ovaries at 90 days of gestation. Ovaries from late pregnancy responded to luteinizing hormone as readily as ovaries from early pregnancy. When luteinizing hormone was added to the citrated bovine blood for perfusion, progesterone synthesis rates increased from 4.37 and 4.59  $\mu\text{g/min}$  to 7.43 and 7.72  $\mu\text{g/min}$  respectively for early and late pregnancy ovaries.

The corpus luteum thus may function throughout gestation and apparently, on the basis of ablation studies, is necessary during late gestation if the cow is to complete a normal gestation period.

Peripheral plasma levels of progesterone in the pregnant cow have been reported for several years. Short (1958)



reported levels of .74 to .98  $\mu\text{g}/100\text{ ml}$  plasma from days 32 to 256 of pregnancy. Thereafter, a gradual decrease in concentration was observed until just before parturition when levels averaged 0.1 to 0.4  $\mu\text{g}/100\text{ ml}$  plasma.

Erb et al. (1968) did not detect a significant change in peripheral plasma progesterone from day 181 through 12 hours postpartum. Progesterone levels ranged from an average of 36 ng/ml during pregnancy to 33 ng/ml at parturition. Similar levels of peripheral plasma progesterone had been reported by Melampy, Hearn and Raker (1959). These workers observed a slight decline during the 250 to 280 day interval. Melampy, Hearn and Raker (1959) and Erb et al. (1968) reported progesterone levels which were three to four times the levels reported by Short (1958) and subsequent investigators.

Pope, Gupta and Munro (1969) assayed peripheral plasma progesterone in normal pregnant and ovariectomized cows by gas chromatography. Plasma progesterone levels ranged from 2.5 to 7.5 ng/ml during the last month of pregnancy and gradually fell to 1.5 to 3.0 ng/ml at calving. Ovariectomized cows had levels below 2 ng/ml.

Donaldson, Bassett and Thorburn (1970); Stabenfeldt, Osburn and Ewing (1970); Arije, Wiltbank and Hopwood (1971); Henricks et al. (1972); Schams et al. (1972); Edqvist et al. (1973) and Smith et al. (1973) found peripheral plasma progesterone levels that averaged 8-10 ng/ml three to four

weeks prepartum. These levels decreased gradually to an average of 5-6 ng/ml the last two weeks of pregnancy and fell very abruptly to 1.5-1.0 ng/ml approximately 24 hours prior to calving. Postpartum levels remained low until ovarian activity was resumed.

The abrupt drop in peripheral progesterone heralds the commencement of parturition in the cow. Holm and Short (1962) reported that Guernsey and Friesian cows with prolonged gestations had no signs of a fall in peripheral progesterone at the expected date of delivery. Hunter et al. (1970) showed that cows with a longer gestation period had higher progesterone levels.

Cows with dexamethasone or flumethasone induced parturitions have a blood progesterone pattern similar to the normal cow. Wright et al. (1970) induced abortion in six of seven cows injected intramuscularly once or twice with 20 mg flumethasone ( $6\alpha$ ,  $9\alpha$ -difluoro- $16\alpha$ -methylprednisolone) between 145 and 245 days of pregnancy. Progesterone levels were 4.2-6.4 ng/ml initially, but these levels declined to 0.5 ng/ml prior to abortion. The one cow that failed to abort did not have a significant lowering of plasma progesterone after treatment nor was there evidence of corpus luteum regression as occurred in the other treated animals.

Edqvist et al. (1972) reported similar findings with dexamethasone induced abortions. Four cows at 248-250 days of

pregnancy delivered live calves 12-68 hours after a second injection of 10 to 20 mg dexamethasone. The peripheral plasma levels of progesterone ranged from about 3 to 10 ng/ml before treatment. A continuous decline occurred in these cows during the last three days preceding parturition. At parturition progesterone levels averaged 1 to 3 ng/ml.

One of four cows pregnant 75 to 150 days aborted after seven daily injections of 40 mg dexamethasone. The corpus luteum decreased in size during treatment as judged by rectal examination. The other three cows failed to show a significant drop in peripheral progesterone with similar treatment levels.

## 2. Estrogens

A comparison of estrogen levels in different mammalian species during pregnancy and parturition is complicated by the variety of sampling and analytical procedures used. In the past plasma estrogens have proven difficult to measure, but newer assay methods utilizing radio-ligand principles are providing more complete information about levels of the steroid.

Urinary estrogens have been utilized in monitoring high risk pregnancies in women including hydrops amnii, placental insufficiency and prolonged pregnancies. Hoag, (1971) reported that urinary estriol levels below 5 mg/24 hours in the last 10 weeks of pregnancy were inconsistent with fetal survival, but no fetal loss occurred if estriol levels were above 16 mg/

24 hours.

In normal pregnancies free plasma estriol, the pre-dominant estrogen in human plasma, averaged 208 ng/100 ml at 9-16 weeks (Munson, Mueller and Yannone, 1970). At term, levels fell from 1196 ng/100 ml to a mean of 423 ng/100 ml by one hour after delivery.

Plasma estrogens have been studied more extensively in the ewe. Because of the dramatic increase at term, estrogens are thought to be the trigger mechanism of ovine parturition (Challis, 1971). Challis, Harrison and Heap (1971a), utilizing radio-immunoassays, reported total unconjugated estrogen levels of less than 5 pg/ml jugular venous plasma for most of pregnancy in the ewe. Within the last 30 days these levels increased to 20-40 pg/ml and rose very sharply during the last 24 hours of gestation to 75-411 pg/ml. Only one animal had detectable plasma estrogen by one day postpartum. Estrone was found to be the major proportion of the total estrogens. Thorburn et al. (1972) and Thompson (1973) reported a similar plasma estrogen increase in late gestation. Their results indicate that the greatest increase came almost entirely during the last 24 hours preceding fetal expulsion. Thorburn et al. (1972) found a mean ratio of 1.9 for estrone estradiol-17- $\beta$  on the day of parturition. Thompson (1973) and Challis, Harrison and Heap (1971a) found that this ratio increased as parturition neared. Therefore as total plasma estrogens increased in late

term, estrone comprised as much as 80% of the total plasma estrogens. Uterine vein samples nearly paralleled peripheral levels. Thompson (1973) found that ovariectomy and adrenalectomy significantly reduced peripheral or uterine plasma estrone levels by 20 to 30%.

Total unconjugated estrogens in the pregnant goat showed a gradual increase from less than 5 pg/ml of plasma during the first 30 days to a high average value of  $622 \pm 77$  pg/ml during the last 10 days of pregnancy (Challis and Linzell, 1971). Plasma estrogen levels fell rapidly after parturition. While multiple births tended to increase maternal plasma levels, there was no significant difference in levels between goats with single, double, or triplet kids.

Thorburn et al. (1972) utilized alumina column chromatography and competitive protein binding methods with a sheep uterine cytosol to separate and analyze maternal and fetal plasma estrogens. They reported that estradiol-17 $\alpha$  was the main estrogen in goat plasma, followed closely by estrone, while estradiol-17 $\beta$  was largely undetectable. In the week preceding parturition, plasma estradiol-17 $\alpha$  ranged from 400 to 1300 pg/ml until a two to threefold increase occurred on the day of parturition. Estrone levels were nearly half the estradiol-17 $\alpha$  levels. Following parturition, peripheral plasma levels of estrogen fell rapidly to 100 pg/ml or less. In one goat estrone and estradiol-17 $\alpha$  were consistently higher

in the uterine vein than in the uterine artery indicating that the placenta is a probable source of plasma estrogen in the pregnant goat.

While urinary estrogen levels have been shown to rise as pregnancy advances in the sow, it was only assumed until recently that plasma estrogens rose in a similar manner. Molokwu and Wagner (1973) measured plasma estrone and estradiol in normal sows from one week prepartum to one week postpartum. Peripheral estrone levels averaged 1200 to 1400 pg/ml plasma during days 7 to 4 prepartum. Thereafter estrone levels increased sharply to a high of 2300 pg/ml 24 hours prior to parturition, and decreased slightly the day of farrowing. Twenty-four hours after parturition estrone fell to about 200 pg/ml and decreased further to nearly undetectable levels by six days postpartum. Estradiol levels in the sow averaged about 1/30 the estrone levels but estradiol levels showed less variation from day to day during this periparturient period.

Urinary estrogens measured by chemical methods have been found to increase throughout pregnancy in cattle. Nelson and Smith (1963) found that the most pronounced increase was in the excretion of estradiol-17 $\alpha$ , although estrone excretion was also considerably higher during late gestation. Estradiol-17 $\beta$  increased at a much slower rate. Mellin, Erb and Estergreen (1966) demonstrated a 29% increase in urinary estrogens during the last 40 hours preceding parturition and a decline of 56%

by 48 hours after parturition. Comparing urinary estrogens in cows with different gestational lengths Hunter et al. (1970) found that cows with longer gestations (over 284 days) had lower total urinary estrogens. Intermediate cows (280 to 284 days gestation) had the highest urinary estrogen levels. Randel and Erb (1971) monitored urinary estrogens from breeding to 260 days gestation. They reported a moderate peak at 42 days and a gradual fourfold increase to day 260.

Using a chemical method (tetrazolium assay) Pope, Jones and Waynforth (1965) reported that bovine blood estrogens, primarily estrone, rose to about 7 ng/ml during late pregnancy. Levels at three months averaged 1-10 ng/l and at five to six months averaged 1 ng/ml.

Robinson, Anastassiadis and Common (1971) measured whole blood estrone in cows before and after parturition with a photo fluorometric method. They observed a peak level of 8.2 ng/ml at day 5 prepartum followed by a moderate decline to day 1 prepartum. A steep decline followed from day 1 prepartum through postpartum. This decline in the last four days prepartum is not consistent with subsequent total unconjugated estrogen or estrone determinations in other studies. Arije, Wiltbank and Hopwood (1971) reported estrogen in the last 20 days ranged from 870 to 1300 pg/ml and fell to 100 pg/ml at parturition.

Henricks et al. (1972) using immunoassay technique reported a mean plasma estrogen concentration of 510 pg/ml at 14 days prior to calving. Plasma estrogen rose to a level of 2660 pg/ml shortly before parturition and fell abruptly thereafter. Estrogen concentrations were consistently higher ( $P < 0.01$ ) in the cows having the shorter gestation period (270-281 days) versus the longer gestation group (285-289 days).

Based on a radio immunoassay, Edqvist et al. (1973) reported that mean plasma estrone concentration ranged from 700-900 pg/ml during the last eight days of pregnancy declining to 100 pg/ml after delivery in six cows. Estradiol-17 $\beta$  levels recorded before parturition ranged from 100 to 250 pg/ml and after delivery the average value was 25 pg/ml. A prepartum decline in plasma estrogen was not detected by Edqvist et al. (1973), but samples were obtained only once per day and not at parturition.

### 3. Corticoids

The importance of a functional fetal adrenal for normal parturition (Drost and Holm, 1968; Liggins, 1968) and the demonstration that exogenous glucocorticoids could induce parturition in the ewe and cow (Liggins, 1969a; Adams, 1969; Adams and Wagner, 1970) have directed attention to the role of corticoids in parturition. In general, plasma corticoids in the dam rise near parturition but this rise generally lags



behind the plasma estrogen increase and the decrease in plasma progesterone.

In the sow plasma cortisol followed a diurnal rhythm averaging 21 ng/ml in the AM and 12 ng/ml in the PM until 24 hours preceding parturition and increasing sharply before parturition (Killian, Garnerick and Day, 1972). Molokwu and Wagner (1973) reported plasma corticoids increased from a mean of 65 ng/ml on days 7 to 2 prepartum to nearly 80 ng/ml 24 hours before parturition and 100 ng/ml at parturition. Plasma corticoid levels fell to near 60 ng/ml by 28 hours postpartum and decreased slightly during the next four days.

Thompson (1973) observed a similar rise in plasma corticoid in the ewe, but the prepartum rise was delayed until the last 24 hours before delivery.

Adams and Wagner (1970) followed plasma corticoids in the cow from seven days prepartum to seven days postpartum. Corticoid levels on day 7, 6 and 5 prepartum averaged approximately 7 ng/ml, while corticoid levels at day 4, 3, 2, 1 or 0 were significantly higher averaging near 13 ng/ml. Postpartum corticoid levels exhibited a gradual decline and were below prepartum levels by day 3. Smith et al. (1973) found total serum corticoids averaged 4.7 ng/ml from day 26 to day 1 before parturition and increased to 10.9 ng/ml near parturition. Levels fell by 12 hours after calving to an average of 4.8 ng/ml and remained near that level in the postpartum period.

In three cows sampled from three to four weeks prepartum through the second postpartum estrus, Arije, Wiltbank and Hopwood (1971) reported corticoids during pregnancy varied from 10 to 50 ng/ml, increasing to 100 ng/ml at parturition. Postpartum levels dropped appreciably and showed considerable fluctuation.

### C. Induction Studies in Domestic Animals

Elective induction of labor in domestic animals has only recently been available in veterinary obstetrics while various drugs are known to stimulate myometrial activity and labor in women (Danforth, 1966; Beazley and Gillespie, 1971).

Early investigative work by Van Rensburg (1967), Liggins Kennedy and Holm (1967) and Liggins (1968) suggested that the pituitary-adrenal system of the fetus plays an active role in the initiation of parturition in the ewe. Van Rensburg (1967) further observed that 60-100 mg cortisol administered daily to the ewe in the last trimester proved lethal to the fetus and a dose of 25 mg after the 140th day precipitated the birth of live lambs within 48 hours. Liggins (1969a) found that the exogenous corticoid must have glucocorticoid rather than mineral corticoid properties to effectively induce parturition.

Adams (1969) demonstrated that cows in the last month of pregnancy can readily be induced to calve with dexamethasone-(9- $\alpha$ -fluoro-16- $\alpha$ -methylprednisolone, DXMS). Parturition

occurred within 22 to 56 hours in 19 of 22 cows after injecting the dam with 20 mg DXMS intramuscularly. Three cows that failed to induce in that time period were at an earlier stage of gestation than most of the others. Prematurity of the calf and retention of placental membranes were the two most common problems. Eighteen of the 19 successfully induced cows had retained placentae. Vulvar edema, filling of the udder and relaxation of the pelvic ligaments developed more rapidly in treated cows. Premature calves were viable but sometimes weak and needed assistance to begin nursing.

Adams and Wagner (1970) reported the successful induction of ewes in late pregnancy with single intramuscular injections of 10 or 20 mg DXMS. The rate of induction failure and the period from injection to fetal expulsion increased in those animals injected in earlier pregnancy. Only two of eight ewes pregnant 133-139 days lambed within 72 hours after dexamethasone treatment, while 9 of 15 ewes pregnant 140-142 days lambed within 72 hours. Adams and Wagner (1970) found that cows were readily induced within 72 hours after treatment. The procedure was quite effective in cows as early as 255 to 264 days of pregnancy. They also reported that plasma corticoids rose from an average of 7 ng/ml to 13 ng/ml the last four days prior to parturition and fell gradually to 2-3 ng/ml by seven days post-partum in normal cows. DXMS treated cows had similar pre-treatment levels of plasma corticoids, but DXMS depressed these

levels to less than 1 ng/ml for 48 to 72 hours after treatment.

Osinga, Stegenga and Jöchle (1971) obtained similar induction results with flumethasone (6 $\alpha$ , 9 $\alpha$ -difluoro-16-methyl-prednisolone) given to cattle in late gestation. Both intramuscular and oral routes of administration were tried, but oral medication resulted in poor and inconsistent induction of parturition.

Hansen and Christiansen (1971), using 20 mg DXMS and Lauderdale (1972a) utilizing 5 mg flumethasone for three days intramuscularly had successful induction in 13 of 16 and 10 of 11 cows, respectively. Lauderdale also demonstrated that 9-fluoroprednisolone was nearly ineffective at this dosage in cows pregnant 268 days. Parturition was induced in 294 Hereford, Angus, Holstein, Brown Swiss, and Crossbred cows by treatment with DXMS or flumethasone (Wagner, Willham and Evans, 1971). A 20 mg dosage of DXMS induced parturition in 159 of 189 cows within 72 hours. The remaining 30 cows calved one week later after a second dose of DXMS. Flumethasone (7.5 mg) or higher dosages of DXMS caused parturition in all treated animals. The average day of gestation was 274.9 days with a range of 267 to 285 days. Placental retention was nearly identical between corticoid treatment groups (54%). The 10 cows that calved without treatment did not retain their fetal membranes. The stage of prematurity and breed of cow affected the incidence of placental retention. Brown Swiss and Holstein

cows had the highest incidence and Angus cows the lowest incidence of retained placentae.

Corticosteroids have been used to induce premature parturition in ewes. Skinner, Jöchle and Nel (1970) injected flumethasone at days 138 to 140 to shorten gestation and improve lamb pelt quality in Karakul sheep. Treated ewes had a mean gestation length of 142 days versus an average of 150 days in control ewes. Bosc (1972) synchronized lambing with 8 to 16 mg dexamethasone given intramuscularly to ewes at 144 days of gestation. These animals responded with parturition in an average of 45 to 47 hours without evidence of detrimental effect to the lambs or to subsequent fertility in the ewe.

The pregnant rabbit also responds to corticosteroid induction of parturition. Rabbits injected on day 27 all had young in less than three days, Adams and Wagner (1970). Placental membranes were not retained.

DXMS (0.34 mg/sac) injected intra-amniotically at 22, 24, 25 or 26 days induced birth in rabbits only if given at day 25 (Kendall and Liggins, 1972). The response was independent of litter size or fetal mortality. The total intra-amniotic dose was usually less than half the 4 mg intramuscular dose given to the doe which was also most effective at day 25. North, Hauser and First (1973) observed that .25 mg/day on days 25, 26, and 27 of gestation or .04 mg transuterine to six or fewer young at one of the above days failed to significantly reduce

rabbit gestational length.

Until recently the pregnant sow and mare were thought not to be responsive to induction of parturition with corticosteroids. Drost (1972) reported that 100 mg DXMS failed to initiate parturition in mares within the last day of pregnancy. Rich et al. (1972) found that 10 or 20 mg of DXMS did not hasten parturition in the sow.

In both the mare and the sow, larger doses of corticosteroids for several days result in premature parturition. Alm, Sullivan and First (1972) injected 12 mares with 100 mg DXMS intramuscularly daily for four days beginning at 320 days gestation. Mean gestation length for treated mares was 328 days versus 340 days for 12 control mares. Most treated mares exhibited normal milk secretion, vaginal dilation and ligament relaxation prior to foaling.

Similarly, 100 mg DXMS given intramuscularly to pregnant sows on days 101, 103 and 104 resulted in early parturition at 109.2 days of gestation (First and Stagmiller, 1972). North, Hauser and First (1973) induced parturition in gilts with 75 mg DXMS daily at 101, 102 and 103 days. These gilts farrowed in 112.6 days compared to 114.7 days in control gilts.

#### D. Control of Parturition

The control of parturition is regulated by a complex interaction of neural, physical and hormonal factors. Certain

facets of this mechanism have been known for several years. Thus the uterine response to oxytocin, the effect of estrogen on myometrial sensitivity to oxytocin and the protective role of progesterone in pregnancy maintenance have been included in the literature for several years.

Subsequently, the role of prostaglandins and the fetal endocrine system have been included as factors influencing the activity of the pregnant uterus. Speigelberg, (1891) cited by Marshall and Moir (1952) advanced the theory that parturition was brought about through the action of substances secreted by the fetus and passed into the maternal blood. He suggested that the exciting substances were elaborated as the result of insufficient nutrition. This theory must be given greater consideration in light of more recent knowledge.

Because of its complexity and the apparent variability between species, the control of parturition in mammals continues to intrigue investigators and has been the subject of many reviews (Reynolds, 1959; Csapo, 1969; Catchpole, 1969; Ryan, 1971; Bedford et al., 1972; Liggins et al., 1972; Chard, 1972). In most of these reviews concerning initiation of parturition the emphasis has been on one or more of the following:

1. A withdrawal of the progesterone block
2. An increase in uterine volume
3. A rise in estrogen levels
4. A change in the progesterone-estrogen ratio

5. An increase in neural responsiveness
6. A release of pharmacologically activated substances (oxytocin, catecholamines, prostaglandins, etc.)
7. An alteration in the relationship of the fetal-maternal endocrine systems

In this review the greater emphasis will be directed towards maternal and fetal steroid hormone levels and their relationship to parturition.

Progesterone is necessary for maintenance of pregnancy in most mammals and it has been hypothesized that its withdrawal induces parturition (Allen and Reynolds, 1935; Csapo, 1956). Progesterone reduced the excitability and contractile activity of the pregnant rabbit myometrium (Csapo and Takeda, 1965). Much of the present knowledge concerning the role of progesterone in pregnancy maintenance is based upon concurrent blood plasma levels during pregnancy and parturition.

In those species which depend upon an extra-placental source of progesterone such as the rabbit, goat, sow or cow there is a marked decline in plasma progesterone a day or two before parturition. The progesterone withdrawal theory is supported by such an event.

In those species where the placenta replaces the ovaries as a major source of progesterone, blood plasma levels at parturition are less consistent and do not totally support the simple progesterone withdrawal theory (Catchpole, 1969). This



is particularly evident in the woman (Llauro, Runnebaum and Zander, 1968) and guinea pig (Challis, Heap and Illingworth, 1971b) where plasma concentrations of progesterone are exceedingly high in late gestation and parturition. Illingworth, Heap and Perry (1970) reported that a 100-fold increase in plasma progesterone was due to a marked decrease in metabolic clearance and an increased production rate. The decreased metabolic clearance rate was related to the production of progesterone binding protein during pregnancy. This is consistent with the finding of Porter (1970) that exogenous progesterone had little effect on the spontaneous or oxytocin-induced activity of the pregnant guinea pig myometrium.

Some workers have shown that intra-amniotic injections of hypertonic saline in women cause plasma progesterone levels to fall prior to abortion (Wiest et al., 1970; Holmdahl, Johansson and Nilsson, 1971). On the other hand Short et al. (1965) reported increased uterine activity and often abortion, before progesterone levels declined.

Ryan (1971) has proposed that plasma progesterone levels may actually overestimate the amount available for biological activity because much of the circulating progesterone is bound to protein.

One such steroid binding protein is transcortin, a corticosteroid binding protein, which increases from the second month of pregnancy to term in women, (Doe, Fernandez and Seal,

1964; Lindner, 1964). Exogenous estrogen elevated serum transcortin in women. Rosenthal, Slaunwhite and Sandberg (1969) demonstrated a significant interplay between plasma progesterone, cortisol and transcortin in women. Unbound cortisol showed a gradual increase during pregnancy, rising to three times its normal plasma levels. Progesterone replaced transcortin bound cortisol increasingly during pregnancy until in the later stages of pregnancy the ratio of bound progesterone to bound corticoid was 0.3. Transcortin bound progesterone may be released near term if transcortin levels are reduced or cortisol levels increased.

Lindner (1964) and Paterson and Hills (1967) found transcortin levels in sheep were about one-tenth of the levels found in pregnant women. Further, transcortin levels did not rise in response to estrogen as they did in women.

Stabenfeldt, Osburn and Ewing (1970) postulated that those animals dependent upon placental progesterone would have little progesterone withdrawal prior to parturition because progesterone production was not independent of the general support of the fetus by the placenta. This appears to be the case in sheep where plasma progesterone levels usually decline just shortly before parturition (Bassett et al. 1969; Fylling, 1971 and Thompson, 1973) which would indicate that placental support continues until parturition is imminent. In this species, plasma and myometrial progesterone levels were similar

near term (Liggins et al., 1972) and decreased in a similar fashion prior to parturition.

Earlier experiments by Csapo and Wiest (1969) showed that the local uterine concentration of progesterone was an important factor in the maintenance of pregnancy. When rats were ovariectomized on the 15th and 16th day of gestation, pregnancy survived even though plasma progesterone levels fell to very low values. Uterine progesterone, apparently provided by placental synthesis, remained above a critical level of about 13 ng/g and parturition did not occur.

Wiest (1970) found that plasma progesterone levels remained fairly high until the 20th day in the pregnant rat and then fell very rapidly. In contrast uterine concentrations declined gradually after a high of 220 ng/g at 11 days to a low of 17 ng/g on the 20th day.

Milgrom and Baulieu (1970) found the level of progesterone in the myometrium was related to progesterone binding proteins in the uterine cytosol of rats. This protein was physiochemically similar to transcortin. Cortisol replaced labelled progesterone more readily than did progesterone in in vitro studies. Davies and Ryan (1973) qualitatively measured the cytoplasmic receptors in the cytoplasm of pregnant rat myometrium. Progesterone receptor sites were found to increase to a maximum at about nine days and to decline by the 12th day. During the last week the concentration of such sites was

roughly 1/4 the peak levels seen at mid-pregnancy. The concentration of receptor sites correlated well with progesterone concentration in the myometrium.

Flint and Armstrong (1973) have demonstrated a 3.5 fold increase in rat endometrial 20 $\alpha$ -hydroxysteroid dehydrogenase during the later half of gestation. This dehydrogenase appeared much earlier in the endometrium than in the ovary where it reaches high levels only during the last one to two days and is probably responsible for decreased ovarian production of progesterone (Wiest, 1970). The increasing level of endometrial 20 $\alpha$ -hydroxysteroid dehydrogenase and decreasing amounts of progesterone binding protein in the myometrium, coupled with the precipitous fall in the level of plasma progesterone could result in a sudden withdrawal of progesterone influence on the pregnant rat myometrium. This may initiate parturition in the rat. However, 5 mg progesterone in oil given two times daily failed to alter the normal termination of pregnancy or the plasma progesterone levels in pregnant rats (Wiest, 1968).

The response to supplemental progesterone for delaying parturition is variable among several other species. Exogenous progesterone fails to prolong gestation in the guinea pig (Zarrow, Anderson and Callantine, 1963; Porter, 1970). Parturition in the rabbit can be delayed with exogenous progesterone or by maintenance of the corpus luteum with estrogen injections (Heckel and Allen, 1939; Csapo, 1956).

Daily administration of 80-160 mg progesterone over the last week of pregnancy did not prolong pregnancy in the ewe, though labor was protracted (Bengtsson and Schofield, 1963). When a single injection of 80 mg progesterone in oil was administered in early labor, parturition was delayed as much as seven days.

Hindson, Schofield and Ward (1969) interpreted the above mentioned results as a compensatory response by the ewe to tolerate high circulatory levels of progesterone. When progesterone is given only at the onset of labor, this compensatory response is not established and parturition is delayed.

The response to progesterone supplementation in glucocorticoid induced parturition is equally variable among species. Liggins et al. (1972) found that exogenous progesterone, 25 to 100 mg/24 hours did not block parturition induced by DXMS infusion in the fetal lamb. With a dose of 100 mg/24 hours, no drop in plasma progesterone occurred. Parturition was inhibited by 200 mg progesterone per day following DXMS treatment to the fetus.

Jöchle et al. (1972) demonstrated that progesterone (100 mg/day) for three days before and five days after 10 mg flumethasone on day 270 of pregnancy prevented premature parturition in the cow. Dystocias and stillborn calves were observed in many of the progesterone treated cows.

In the pig, DXMS induced parturition can be successfully blocked by exogenous progesterone (First and Stagmiller, 1972).

It appears that progesterone can block natural or glucocorticoid induced parturition in those animals which normally have a marked decline of progesterone prior to parturition. This is particularly true in the cow, goat and sow which are dependent upon an ovarian source of progesterone in late pregnancy.

Csapo (1969) suggested that the mechanical stimulation of the uterus as it stretches with increasing volume has a significant influence on parturition. This may be particularly evident in humans where twin pregnancies or hydramnics precipitate early parturition. This modified progesterone block theory is based upon a uterine volume:progesterone ratio. This theory suggests that the amount of progesterone available per unit of uterine tissue decrease until a critical point is reached when it no longer suppresses uterine activity. Bedford et al. (1972) contended that this gradual change is unlikely to result in precise timing associated with the initiation of parturition.

It is highly probable that the rising levels of plasma estrogens in the dam are associated in some manner with triggering parturition. This group of steroid hormones have profound effects on both the pregnant and non-pregnant uterus.

They increase blood flow and vascular permeability, increase electrical conductance of the myometrium and increase myometrial response to oxytocin (Csapo, 1969). Estrogen has an anabolic effect on energy formation, particularly ATP and ADP accumulation, within the myometrial cells (Vorherr, 1968).

There is evidence that the administration of estrogen can induce parturition in some species near term. Hindson, Schofield and Turner (1967) found that a single injection of 20 mg stilbestrol caused increased uterine activity within 24 hours and parturition within two days after injection in four of six ewes, 136 to 142 days pregnant. However, several of the sheep suffered prolonged labor due to cervical dystocia.

Gronborg-Pedersen (1969) induced parturition in 10 of 13 cows in late pregnancy with 10 to 25 mg estradiol benzoate. These cows responded by calving in one to two days after intramuscular injection. Seven of the ten cows had retained placentas. In contrast Adams (1969) reported that estrogens given at pharmacological doses failed to initiate parturition in the cow.

In the rat the estrogen rise prior to parturition is of ovarian origin. Ovariectomy in the last two days of gestation delays parturition unless exogenous estrogens are supplied (Csapo, 1969).

There is good evidence that a prepartum rise of urinary or plasma estrogens occurs in all species. The ewe provides

the best evidence for estrogen as the initiator of parturition. Maternal circulating estrogens peak within the last 24 hours prepartum in this species (Challis, 1971).

Liggins et al. (1972) disagree with Challis' premise that estrogen may provide the trigger for parturition. They failed to find a significant rise in plasma estradiol-17 $\beta$  in some ewes which were induced by ACTH or dexamethasone administration to the fetal lamb. This was particularly evident in ewes who were less than 130 days of pregnancy.

Bedford et al. (1972) summarized the possible contributions of estrogens in controlling parturition:

1. They may act directly upon the myometrium to overcome the progesterone block by estrogen dominance. This is in general agreement with the proposal that an increase in the estrogen:progesterone ratio sensitized the uterus to the effect of oxytocin (Catchpole, 1969).

2. Estrogens may act to increase the release of oxytocin in response to vaginal distension. This has been shown to occur in non-pregnant sheep (Roberts and Share, 1969) whereas the opposite effect occurred after progesterone treatment.

3. Estrogens may trigger the release of prostoglandin F<sub>2 $\alpha$</sub>  from the uterus. Blatchley et al. (1971) demonstrated a 300% increase in prostaglandin F<sub>2 $\alpha$</sub>  in the uterine vein after three days of estradiol treatment in cycling guinea-pigs.



Estrogen levels, both urinary and plasma, rise gradually over the last two trimesters of pregnancy in most mammals. This increase is accentuated as term approaches and is related to the ability of the fetus to produce estrogen precursors. Within the last 24 to 48 hours there is a two to threefold increase in plasma estrogens. This may reflect an increased fetal adrenal activity, whereby both estrogen precursor and glucocorticoid production increases. A second possibility for the sudden increase in estrogens in late term is the conversion of greater proportions of estrogenic sulfate conjugates to free estrogens.

The physiological effects of estrogen upon the myometrium include an inter-relationship with oxytocin. Oxytocin stimulates myometrial activity by lowering the membrane potential and threshold of the myometrial cells. Csapo and Sauvage (1968) noted an increase in sensitivity of the uterus to oxytocin as pregnancy progressed, but the greatest sensitivity occurred at term just before and during labor. The sensitivity of the myometrium to oxytocin is enhanced by estrogens and depressed by progesterones (Roberts and Share, 1969).

During parturition the stimuli for oxytocin release arise primarily from mechanical stimulation of the uterus, cervix and vagina. Oxytocin levels in human maternal blood during labor are low at the first stage and higher during the second

stage. However, Chard (1972) found that plasma levels fluctuated greatly at all stages of labor and that there is no relationship to uterine contractions or any large increase at the time of delivery; rather, oxytocin release occurs in short acting spurts. Chard et al. (1970) and Fitzpatrick and Walmsley (1965) observed that plasma oxytocin levels in the cow, mare, goat and sheep show only small changes during labor but rise to a peak at the actual moment of delivery and decline rapidly thereafter. In rabbits, the uterine response to oxytocin increased very markedly within 48 hours of parturition (Fuchs, 1964). Transections of the spinal cord in the thoracic region did not prevent the onset of labor in the rabbit, but fetal death and desultory labor were common (Beyer and Mena, 1970).

Fuchs and Poblete (1970) observed uterine response to oxytocin in pregnant rats by intra-uterine balloons. Parturition could not be induced earlier than four to six hours before expected term although uterine contractions were elicited by oxytocin infusions several days before term. At parturition the uterine contractions were synchronous, whereas before term both ends of the uterine horn contracted independently.

More recently Chard et al. (1971) found considerable oxytocin and vasopressin in the human fetal umbilical plasma. Arterial levels were higher than venous, suggesting a fetal pituitary source. Fetal oxytocin levels varied a great

deal during labor and appeared not to correlate well with uterine contractions. It does suggest, however, another role that the fetal pituitary may play in its own delivery. In a recent review Chard (1972) concluded that the function of oxytocin was to expedite the process of delivery and that it was unlikely to be solely responsible for the onset of labor.

More recently prostaglandins have attracted attention because of their possible association with pregnancy maintenance and parturition. This group of related 20 carbon hydroxy-fatty acids are widely distributed in mammalian tissue. Karim (1968) reported the presence of prostaglandins  $E_1$ ,  $E_2$ ,  $F_{1\alpha}$  and  $F_{2\alpha}$  in human amniotic fluid. A relationship between prostaglandin (PG) content of amniotic fluid and labor was established. Prior to labor only  $PGE_1$  and  $PGE_2$  were detected in very low concentration. Samples obtained during labor contained high concentrations of PGF compounds.  $PGF_{2\alpha}$  was also present in maternal venous blood. Concentrations of prostaglandins  $E_1$ ,  $E_2$ ,  $F_{1\alpha}$  and  $F_{2\alpha}$  in the decidua from patients in labor were 10 to 30 times higher than in amniotic fluids.

Liggins and Grieves (1971) measured the concentration of prostaglandins in placental tissues of ewes before and during premature parturition induced by fetal infusion of ACTH or glucocorticoids.  $PGF_{2\alpha}$  was found in the uterine vein plasma but only after labor commenced. The concentration of  $PGF_{2\alpha}$  increased in the myometrium and maternal cotyledons with the advent of labor, but fetal cotyledons showed no change.

Prostaglandins  $E_2$  and  $F_{2\alpha}$  have been used to induce labor in midterm, late term and term women. Various routes of administration are equally effective with proper dosages: Intravenously, (Csapo, Sauvage and Wiest, 1971a; Karim, 1972); Intrauterine, (Embrey and Hillier, 1971); Orally, (Barr and Naismith, 1972); and Intra-amniotic, (Anderson, Hobbins and Speroff, 1972b).

Prostaglandins given intravenously were as effective as oxytocin in term patients (Anderson, Hobbins and Speroff, 1972b; Beazley and Gillespie, 1971; Bygdeman, Roth-Brandel and Wiggvist, 1970). The latter group found that uterine sensitivity to prostaglandins is particularly independent of the stage of pregnancy. This is in distinct contrast to oxytocin, where the sensitivity of the uterus to oxytocin is low during early stages of pregnancy.

These products are currently receiving considerable attention in animal reproduction. They have been shown to terminate luteal activity in rats, hamsters, mice, rabbits, guinea pigs, and sheep (McCracken, Glew, Scaramuzzi, 1970; Thorburn and Nicol, 1971; Pharriss, Tillson and Erickson, 1972; Labhsetwar, 1972). Prostaglandins do not appear to be luteolytic in humans from the seventh week of gestation on (Speroff et al., 1972). Plasma progesterone and estrogens were not significantly altered prior to  $PGF_{2\alpha}$  abortions. However, Csapo, Sauvage and Wiest (1971a) had previously reported

a decrease in both plasma estradiol-17 $\beta$  and progesterone prior to mid-pregnancy abortions following PG treatment. Lehmann et al. (1972) demonstrated a luteolytic effect in a non-gravid woman with PGF<sub>2 $\alpha$</sub> . In several patients hospitalized for therapeutic abortion (10-18 weeks gestation) PGF<sub>2 $\alpha$</sub>  infusions caused a pronounced decline in progesterone levels in most cases. It was concluded that the abortifacient action of PGF<sub>2 $\alpha$</sub>  could be attributed to its effect on the uterus and consequent disruption of the placenta. The situation may be somewhat different in term pregnancy since LeMaire et al. (1972) found that neither oxytocin or PGF<sub>2 $\alpha$</sub>  infusions altered plasma levels of progesterone or estriol in term women.

Prostaglandins are also abortifacients in animals. Cows pregnant 40 to 180 days were aborted with 45 and 150 mg PGF<sub>2 $\alpha$</sub>  tam salt in multiple or single intravenous or subcutaneous injections (Lauderdale, 1972b). All of the twenty cows aborted in two to seven days. Doses of 15 or 30 mg were less effective. Liggins et al. (1972) demonstrated that PGF<sub>2 $\alpha$</sub>  could terminate 110 to 140 day pregnancies in ewes. Free estradiol-17 $\beta$  levels rose rapidly while progesterone levels declined slightly within the first 16 hours after intra-aortic infusion of prostaglandin began. Only high doses of progesterone, at least 200 mg/day, could prevent abortions. In the rabbit myometrial activity induced by prostaglandins can be inhibited by progesterone (Porter and Behrman, 1971).

It is apparent that prostaglandins have an oxytocin-like effect upon the myometrium, particularly in women, but its mechanism of action in luteolysis is still uncertain. Speroff and Ramwell (1970) and March (1971) found that  $\text{PGF}_{2\alpha}$  stimulated in vitro steroidogenesis and progesterone production in bovine corpus luteum slices. This appeared to be mediated through an increase in adenyl cyclase activity. However, Pharriss and Wyngarden (1969) infused  $\text{PGF}_{2\alpha}$  into pseudo-pregnant rats and noted an increased shift from progesterone to a  $20\alpha$ -hydroxy-progesterone.

The mechanism for PG stimulation and release at term is still uncertain. Bedford et al. (1972) considered the possibility that estrogens triggered the release of  $\text{PGF}_{2\alpha}$  from the uterus of the sheep. In ovariectomized non-pregnant ewes PG levels rose after a sequence of progesterone and then estradiol injections (Caldwell et al., 1972). Estrogen treatment in the non-pregnant guinea pig stimulated the release of  $\text{PGF}_{2\alpha}$  from the uterus (Blatchley et al., 1971).

Liggins et al. (1972) conceded the possibility of estrogen stimulating PG release in the term ewe, but offered strong evidence that fetal corticosteroids stimulate PG synthesis and release.

In summary it appears that fetal corticoids rise significantly in response to maturation of the fetus near term. In addition there is a prepartum rise in urinary and plasma

estrogens and a concomitant decrease in progesterone in most domestic species but not in the human or guinea-pig. Most authorities agree that prostoglandins, particularly  $\text{PGF}_{2\alpha}$ , may serve a dual function, being luteolytic in some species and myometrial stimulants in others. At present the mechanisms resulting in the various steroid changes and their interactions in triggering parturition are not resolved.

#### E. Postpartum Reproductive Function

##### 1. Postpartum fertility

Reproductive efficiency in the cow is dependent upon maintenance of a desirable calving interval. Postpartum fertility has a decided effect on maintenance of a normal calving interval. For this reason, considerable attention has been focused on conception rates and the optimum time of breeding the postpartum cow.

Most reports indicate that conception rates increase as the postpartum interval lengthens up to day 50 to 60 (Edwards, 1950; Van Denmark and Salisbury, 1950; Shannon, Salisbury and Van Demark, 1952). After 60 days postpartum conception rates plateau. Studies in beef cows indicate that return of optimum fertility is near 60 days (Warnick, 1955; Perkins and Kidder, 1963; Foote and Hunter, 1964). Perkins and Kidder (1963) concluded that conception rates were not affected by the involutionary state of the uterus as judged by rectal palpation.

The consensus of these reports indicate optimum fertility is reached in both dairy and beef cows sometime between 50 and 75 days postpartum. In the past half century, researchers have quested to delineate the factors that influence postpartum fertility. Most of the research has been done in the last two decades, but rather acute observers, such as Hammond (1929), provided considerable knowledge in the area of bovine reproduction.

Most of the basic work in postpartum physiology is based on visual observation or rectal palpation. This probably allows for the considerable variance seen in reports from different authors. More recent reports consider the factors affecting ovarian function and uterine involution, such as anemia, exogenous hormones or the relationship of thyroid, pituitary and adrenal function to reproductive activity.

## 2. Follicular growth

Early follicular development and ovulation by 16-21 days after parturition was reported by Hammond (1929). More recently, Labhsetwar et al. (1964) examined by slaughter 20 multiparous Holstein cows at 260 to 265 days of gestation, at parturition, and at 21 days postpartum. Three of five cows had already ovulated by 21 days but estrus was observed in only two.

Wagner and Hansel (1969) found the average size of the largest follicles present at 7, 14 and 30 days postpartum were



9.6, 11.3 and 13.1 mm in diameter respectively. There was no difference in follicular size at 30 days postpartum between cows milked twice daily, nursed cows and anemic cows.

Comparing follicular size in postpartum milked, suckled and non-lactating cows, Wagner and Oxenreider (1971) found that suckling tended to delay the occurrence of a follicle greater than 10 mm in diameter from nine days in non-lactating cows and 13 days in milked cows to 16 days in suckled cows. In general this agrees with Saiduddin et al. (1968) though he found that lactation did not delay follicular development.

### 3. First estrus and first ovulation

Numerous studies indicate that the interval from parturition to first ovulation is shorter than the interval to first estrus. The time of first ovulation based on rectal palpation (Morrow et al., 1966) or observation and slaughter (Wagner and Hansel, 1969) appears to be approximately 14-15 days. In contrast intervals from parturition to first estrus generally range from 32 days (Menge et al., 1962) to 60 days (Casida and Wisnicky, 1950). The incidence of ovulations not associated with an observed estrus appears to be about 68% (Menge et al., 1962). Morrow et al. (1966) reported that this phenomenon was most prevalent at the first ovulation and decreased as each cycle occurred.

Suckling generally delays the resumption of normal reproductive cycles in the dam (Hammond, 1929; Clapp, 1937).

Reviewing the reproductive performance of a herd of milking Shorthorn, Wiltbank and Cook (1958) found a significant difference in return to normal reproductive cycles between milked and nursed cows. Cows milked twice daily had their first postpartum ovulation at an average of 36 days and their first estrus at 54 days. In comparison, nursed cows averaged 53 days until ovulation and 85 days until first estrus. Nursed cows had a significantly poorer conception rate, 1.84 services per conception versus 1.54 services for milked cows ( $P < 0.01$ ).

Summarizing the results of 18 studies Morrow, Roberts and McEntee (1969) found that normal dairy cows averaged 49.8 days to first estrus while nursed dairy and beef cows averaged 68.0 days.

Citing many of the above mentioned references, Casida (1968) summarized the findings of 26 studies. The interval from parturition to first succeeding estrus and first ovulation were compared in dairy cows, beef cows and various milking managements as well as suckled cows. The average length for the different studies on dairy cows ranged from 30 days to 72 days. Comparable figures for the beef cows were 46 to 104 days. The interval from parturition to first succeeding ovulation ranged from 20 to 45 days for dairy cows and 36-71 days for beef cows.

More recently Wagner and Oxenreider (1971) observed that suckled cows averaged 52 days to first ovulation and 56 days for first estrus. Less than half of the nursed cows had shown estrus by 56 days. Non-lactating cows averaged 24 days to first ovulation and 41 days to first estrus.

Oxenreider (1968) showed that removal of the effects of nursing, or adding gonadotropin therapy, shortened the intervals to first ovulation and first estrus in a study involving 25 multiparous Aberdeen Angus cows. No difference was noted between the treated cows and control nursed cows in average time of conception within 100 days after calving.

Short et al. (1972) compared the postpartum performance of suckled, milked and mastectomized cows. Mastectomy was performed prior to 150 days gestation and feeding was adjusted to requirements for maintenance and lactation. The average interval from calving to first estrus was 54, 30 and 14 days, respectively, in suckled, non-suckled and mastectomized cows. Conception occurred at an average interval of 61, 50 and 44 days in these same cows. Removing calves at birth shortened the postpartum interval and mastectomy shortened the interval even further.

Citing unpublished data, Short et al. (1972) demonstrated that 40 units of oxytocin three times per day for 35 days following calving, did not lengthen the postpartum interval in intact or mastectomized cows.

Several excellent review manuscripts concerning post-partum involution and ovarian activity are available (Casida, 1968; Morrow, Roberts and McEntee, 1969; Wagner and Oxenreider, 1971).

#### 4. Uterine involution

Uterine involution by definition means a return of the uterus to normal size after parturition. The criteria used most frequently in clinical studies to determine the degree of involution are a return of the uterus to a normal position, tone, consistency and size approximating the non-pregnant condition. A majority of the reports in the literature are subjective data based on rectal palpation at various time intervals of examination.

Buch, Tyler and Casida (1955) reported the interval of uterine involution as 42 days in primipara and 50 days for pluripara based on 322 parturitions with weekly rectal palpations. Involution was shortest (44 days) in the summer months. Foote, Hauser and Casida (1960), in a similar study involving 13 pairs of twin Herefords, found an interval of 43.7 days for uterine involution. Menge et al. (1962) reported uterine involution was complete at 42.3 days as determined by rectal examination. The authors concluded that the process of uterine involution appeared to be largely independent of ovarian activity.

An interval of 37.7 days was reported by Perkins and Kidder (1963). This study involved rectal palpation of 255 service periods in Hereford and Angus cows. They concluded that the conception rate was not affected by the involutionary state of the uterus as judged by rectal palpation. Tennant, Kendrick and Petticord (1967) in analyzing 2338 genital organ examinations, agreed with Rasbech (1950) stating that involution was complete within 25 to 26 days. Their data did not suggest a significant difference between pluripara and primipara. Morrow et al. (1966) palpated 208 dairy cows twice weekly and reported that uterine involution was complete by 25 days, but periparturient diseases delayed involution an average of at least five days.

Rasbech (1950) further defined involution as two simultaneous processes that culminate in a return to normal estrual and secretory cycles. He classified the two processes as functional involution and placental involution. Functional involution as described by Rasbech is contraction and retraction of the Tunica muscularis. By 8-10 days postpartum the entire uterus was definable by rectal palpation and involution completed by 18 to 20 days in primipara and 20 to 25 days in pluripara. The involuting gravid horn never reached the pre-pregnancy size and remained just slightly thickened, according to Rasbech.

Placental involution, the dissolution of the maternal placenta, is dependent upon regressive tissue changes. Rasbech describes placental involution as three partially coordinated processes comprising regression of the caruncular stalk, dissolution of the decidua and formation of uterine lochia.

Vasoconstriction resulted in shortening of the caruncular stalk. This process was complete by nine days postpartum. Vasoconstriction also resulted in dissolution and detachment of the decidua by day 10. Uterine lochia was largest (1400-1600 ml) at one to two days and decreased to 500 ml at day 8. Only a few ml of uterine lochia was present in the normal cow by day 14-18. The lochia was an admixture of decidual detritus, blood and mucus from the cervix. Epithelial cells of the maternal placenta, the mucosa and the endometrial glands together with histogenic cells, mast cells and plasmacytes, all in advanced stages of regression, were found to be mixed with erythrocytes and white blood cells.

Puerperal infections occurred in clinically normal cows with a frequency of 25-30% in Rasbech's study. This resulted in more lochia near the end of the puerperium. It was Rasbech's conclusion that the normal regressive process was complete in 20 to 25 days.

Gier, Singh and Marion (1962) reported the result of a histological examination of 131 uteri from cows 3 to 65 days postpartum. They observed that neutrophils accumulated in the

sloughing placenta mass and to a lesser extent in the stratum compactum during the first 15 days. Histiocytes were numerous in the caruncular stratum compactum and stratum spongiosum, reaching a maximum by 40 days and were usually gone by 60 days. Neutrophils were numerous in infected cows beyond 30 days postpartum. Lymphocytes were also numerous in infected uteri, but only moderate in sterile uteri. Lymphocytic nodules were found in most uteri with persisting bacterial infection. Mast cells, eosinophils, and monocytes were reported as occurring irregularly in the postpartum uterus.

Uren (1935) examined the involuted uteri of 18 ewes killed at various times from a few hours after parturition to 30 days postpartum. His description of caruncular involution in the ewe was similar to Rasbech's (1950) description in the cow. A zone of hyaline degeneration was seen at the caruncular surface and involving the base of the crypts. By the seventh day postpartum all the cells of the crypts were necrotic. Bits of necrotic fetal placenta were also seen. Exfoliation and liquifaction were described as the process of reducing the necrotic crypt mass, which was completely eroded by day 13. Leucocytes were most numerous at the 11th day. Proliferation and spreading of the glandular mucosa resulted in re-epithelization of the caruncular surface. This process was completed by 26-30 days postpartum.

Sawhney, Quevedo and Foote (1966) compared the histological changes in the uterus of intact and spayed postpartum cows at day 1, 20 and 70. Structure and height of the endometrial epithelium and number of endometrial glands were the primary parameters evaluated. Caruncular epithelial cells were higher in the day 20 operated group than intact or 70 day operated cows. The authors concluded that ovariectomy did not have a significant influence in the uterus during early postpartum anestrus.

Gier and Marion (1968) studied the macroscopic and microscopic changes in the uteri of 57 clinically normal postpartum dairy cows. Macroscopically they found the postpartum uteri decreased in size in a decreasing logarithmic scale. The uteri contracted to half of the gravid diameter at about day 5 and a half of the gravid length at 15 days. By 30 days the postgravid horn was a third of its former gravid size and normal involution was complete by about day 50. The average weight of the uterus decreased from 9.0 kg at parturition to 1.0 kg at 30 days, and to 0.75 kg at 50 days. Their data disagreed with Morrow et al. (1966) in that a fairly uniform rate of uterine regression was found from day 5 to 15.

Rapid myometrial contractions and endometrial edema during the first few days postpartum results in a differential reduction of the uterine layers. The resulting enfolding of the endometrium was considered to represent the "stalked"



caruncle described by Rasbech (1950). Endometrial edema had receded by the sixth and eighth days and the endometrium decreased more rapidly than the myometrium. Caruncular blood vessels constricted rapidly and were nearly occluded within two days. Necrosis of the surface layer of the caruncle progressed in much the same manner as described by Rasbech (1950) and Uren (1935). Organized lymphocytic nodules were observed within the stratum compactum of all postpartum uteri that carried infection and in many uteri 10 to 50 days after parturition from which cultures were negative.

Regeneration of the uterine epithelium began almost immediately after parturition. The entire intercaruncular luminal surface appeared normal by eight days after parturition. If bacterial infection occurred during the period of tissue loss, the epithelium was again partially or completely destroyed.

Rasbech (1950) concluded that re-epithelization of the caruncle was established by 20 days postpartum while Gier and Marion (1968) found re-epithelization was not complete until day 25. The caruncle surface was found to have shrunk to 5 to 8 mm by 40 to 60 days. All phases of uterine involution were noticeably delayed by secondary uterine infections, retained placenta or poor physical condition in the dam.

Riesen et al. (1968) studied the effect of suckling on the macroscopic and microscopic changes of involution.

Involution was observed over three periods; 1 to 10, 10 to 20 and 20 to 30 days. The myometrium was observed for muscle cell size and number and the endometrium was observed for cell type and regressive changes. The rate of uterine involution was more rapid in suckled than in non-suckled cows during the 1-10 and 20-30 day periods. This resulted in the suckled animals being nearly involuted by 30 days while the non-suckled animals were not involuted at either the 30 day postpartum or during the first estrus cycle.

In agreement with Rasbech (1950), Riesen et al. (1968) reported that phagocytic cells (histiocytes) were found to increase during the first 30 days, while lymphocyte numbers increased up to 20 days, and polymorphonuclear cells generally decreased after 10 days postpartum.

Wagner and Hansel (1969) reporting on the clinical and histological finding in the postpartum cow, found that neither suckling nor anemia delayed uterine involution. Histologically, their results agree closely with those reported by Gier and Marion (1968). Giant multinucleated cells of unknown origin were reported by Wagner and Hansel (1969). The nuclei were usually located peripherally in these cells and appeared to be distinct from the large binucleated trophoblastic cells seen in some species during pregnancy. The authors also described the process of regeneration. In some areas the surface epithelium appeared highly proliferative and anaplastic with

vacuoles present in the cytoplasm at 14 days postpartum. Five of the 21 animals showed scattered foci of lymphocytes in the stratum compactum but these foci were not related to the degree of regeneration. Uterine mucosal epithelium was re-established in most of the normal cows within 30 days postpartum.

Wagner and Hansel (1969) found the endometrial glands to be more developed in milked 30 day cows as compared to anemic or nursed 30 day postpartum cows. They attributed this difference to the increased incidence of ovulation and corpora lutea formation in the milked cows. They concluded that normal cyclic ovarian activity was not essential to the overall process of involution. This conclusion is in agreement with several other reports (Menge et al., 1962; Sawhney, Quevedo and Foote, 1966; Oxenreider, 1968).

##### 5. Exogenous hormone treatments

Several investigators have sought to shorten the postpartum interval with exogenous hormone therapy. Cameron and Fosgate (1964) administered 200 units of oxytocin daily for 42 days after parturition. They noted no significant difference in the interval to the first estrus, first ovulation or uterine involution. Short et al. (1972) showed that 40 units of oxytocin three times per day for 35 days did not alter the postpartum interval in either intact or mastectomized cows.

Casida and Wisnicky (1950) administered 20 mg diethylstilbestrol to 60 dairy cows within nine hours after parturition. No effect on postpartum function was evident. Foote and Hunter (1964) demonstrated that 10 mg estradiol in corn oil given intramuscularly on day 25 shortened the postpartum interval. In addition estradiol with progesterone (50 mg subcutaneously daily from days 12 to 25) or progesterone alone also shortened postpartum intervals. Conception occurred earliest in cows given both hormones.

Marion, Norwood and Gier (1968) found that neither ovariectomy nor physiological levels of  $17\beta$ -estradiol given throughout the involution interval, significantly influenced the regression rate of the uterus. However, 30 mg progesterone each day was found to increase the involution interval in both intact and ovariectomized cows.

Oxenreider (1968) and Foote et al. (1966) demonstrated the responsiveness of ovaries to exogenous gonadtropic hormones. Such treatments generally shortened the interval to first estrus and first ovulation, but did not appreciably affect uterine involution.

Kudlac and Benysek (1969) have shown that glucocorticoids (flumethasone 2.5 mg, intramuscularly or 5 mg orally) given on days 4 or 10 after parturition shortened the service period by 11 days. First estrus occurred six days sooner in these cows

also. No estimation of uterine involution was attempted and all cows were given one gram chlortetracycline intrauterine on the first day after calving.

### III. MATERIALS AND METHODS

#### A. Experimental Design and Animals

##### 1. Experiment I

Twenty-three primipara cows were induced near term with Dexamethasone<sup>1</sup> (DXMS). Average gestation length was 281 days with a range of 269 to 299. Eleven Holstein and 12 Brown Swiss heifers comprised the group. They were provided adequate nutrition with corn silage in addition to pasture while nursing their calves. No uterine or systemic medication was given nor was manual removal of retained fetal membranes attempted.

Uterine involution and ovarian activity was evaluated by rectal palpation at two, four and six weeks postpartum. A uterine biopsy sample was taken of each cow at the same examination. Biopsy specimens were obtained with a Yoeman biopsy punch from the prior pregnant horn, just anterior to the uterine bifurcation. A few cows developed uterine wall abscesses after sampling; they were not biopsied thereafter.

Twenty-three cows were biopsied at two weeks, 22 at four weeks and 15 cows were biopsied at six weeks. Biopsy samples were fixed in Bouin's solution and saved for histological examination.

---

<sup>1</sup>9 $\alpha$ -fluro-16 $\alpha$ -methyl prednisolone.

## 2. Experiment II

The objectives of this study were to determine blood plasma levels of certain hormones and the rate of uterine involution in the cow following dexamthasone<sup>1</sup> (DXMS) induced parturition. Eight grade Holsteins and one Brown Swiss, part of Iowa State University's veterinary teaching herd, were selected for induction studies. These cows were confined to stanchions until calving and fed hay and concentrates sufficient to maintain good body weight. Pregnancy was confirmed at 35 to 45 days following natural service to a Hereford bull. The pregnant horn and its corpus luteum was determined and recorded. In each animal the corpus luteum was located ipsilateral to the pregnant horn. An additional cow, number 20, was not induced nor slaughtered, but served as a surgical control animal.

Induction of parturition was attempted at 270 to 274 days of pregnancy. Twenty mg of DXMS were administered intramuscularly as described by Adams (1969).

Indwelling vinyl catheters (.058" ID. x .080" OD)<sup>2</sup> were placed in the jugular vein of each cow. Blood samples were collected at eight hour intervals for two to seven days prior to induction. Immediately prior to glucocorticoid treatment, indwelling vinyl catheters (.034" ID x .057" OD)<sup>2</sup> were placed

---

<sup>1</sup>9 $\alpha$ -fluro-16 $\alpha$ -methyl prednisolone.

<sup>2</sup>Becton-Dickinson, Rutherford, N.J.

in the anterior uterine vein of the pregnant horn of cows 38, 15, 22 and 23. The surgical approach was a standing flank laparotomy. Catheters were positioned distal to the ovary so ovarian effluent was not sampled. An additional catheter was placed in the anterior uterine vein proximal to the ovary in cows 22 and 23. This allowed for collection of blood containing both ovarian and uterine effluent. Catheters were passed through a thin walled 12 gauge needle inserted in the anterior uterine vein. The needle was withdrawn and the catheters sutured to the broad ligament with 00 silk. The free ends of the catheters were exteriorized through the incision, fitted with metal adapters and flushed with 0.9% saline containing 400 units of heparin per ml. The catheters were sutured to the upper flank and the laparotomy closed in three layers. Cows 20 and 24 were subjected to laparotomy but internal catheterization was not successful.

A blood sample was collected from all sample sites following surgery and prior to DXMS treatment.

Following parturition, the cows had free access to their calves during the entire postpartum interval. Retained fetal membranes were recorded, but manual removal and medications were avoided. Rectal palpation was not attempted during the postpartum period to avoid any influence on uterine involution. Cows were observed daily for estrus and ran freely with the bull. The eight cows that were successfully induced were



slaughtered at 14, 28 or 42 days postpartum. Three cows were to be slaughtered at each interval but cow number 38 did not calve and was not slaughtered.

Two additional cows were used to study steroid hormone changes but were not slaughtered nor studied for uterine involution. Cow number 2, a Holstein was induced at approximately 250 days gestation and cow number 1, a Jersey, was treated at approximately 270 days. These cows were subjected to chronic catheterization of the jugular vein prior to surgery for placement of catheters in the anterior uterine vein. Uterine and utero-ovarian blood was collected hourly following DXMS treatment.

Twelve ml of blood were collected from each catheter at eight hour intervals pretreatment and hourly after DXMS treatment. After each collection five ml of heparinized saline was instilled into each catheter to prevent blockage of the catheters. The heparinized saline was withdrawn and discarded before the next blood sample collection. Residual amounts of heparin within the catheter and syringe served as the anti-coagulant for the next blood collection. Blocked catheters were filled with thrombolysin<sup>1</sup> for one to two hours. A few catheters remained blocked and sampling of this site ceased.

---

<sup>1</sup>Merck and Co., Inc., West Point, Pa.

Collected blood was centrifuged immediately to remove the plasma. The plasma was divided into 2 ml aliquots, identified, frozen, and stored at -20 C until thawed for hormonal analysis.

As soon as possible after slaughter, the reproductive organs and endocrine glands were removed. The uterus was trimmed of its mesometrium and the diameter of each horn measured at the bifurcation. Uterine content and amount was noted as each uterine horn was dissected and weighed. Oviducts were dissected and their lengths measured. In similar manner the cervix was freed and its weight and length recorded.

The ovaries were weighed and cyclic corpora lutea were removed, measured and weighed. A small section of corpus luteum was placed in 95% ethanol to be frozen and stored for possible progesterone analysis. Ovarian follicles were counted and measured and the number over 1 cm recorded.

Three sections of each uterine horn, two of which contained portions of the caruncular surface, were fixed in Bouin's fixative. The ovaries and portions of the cervix and vagina were also fixed. Following fixation each ovary was cut in 2-3 mm slices and cross sections of the corpus luteum of pregnancy measured.

The pituitary was removed, weighed, and divided into anterior and posterior portions. A medial section of the anterior pituitary and the entire posterior pituitary were fixed in Bouin's fixative. The remaining portion of the

anterior pituitary was frozen for possible assay of hormones. Special fixatives and stains of the pituitary were not used in this study.

The adrenals were removed, trimmed and weighed. A small section was removed for histological examination. The remaining portion was frozen in 95% ethanol for possible analysis of corticoids and progesterone.

In like manner the thyroid was removed, weighed and fixed for histological examination. No other tissues were collected from these cows.

### 3. Experiment III

Seven Hereford cows belonging to the University veterinary teaching herd were pretreated with human chorionic gonadotrophin (HCG)<sup>1</sup> before attempted induction with DXMS. These cows were in late gestation, and exhibited udder swelling, vulvar swelling and relaxation of the pelvic ligaments. Breeding dates were approximated; therefore induction was not attempted until these signs were evident. All seven animals were catheterized in the jugular vein only. Samples were drawn every eight hours until parturition occurred. After the first 24 hour sample period, six cows were treated with 1000 units HCG at each sample collection for three days. Twenty mg DXMS

---

<sup>1</sup>Follutein - E. R. Squibb & Sons, Inc., New Brunswick, N.J.

was administered by intramuscular injection within minutes after the sixth sample collection, which was 24 hours after HCG treatment had been initiated. One of the seven cows was sampled as a control animal. Sample collection and handling was performed in the same manner as previously described. Samples were assayed for plasma progesterone and estrogen levels, but corticoid levels were not determined.

## B. Steroid Assays

### 1. General procedures

Competitive protein binding techniques as described by Murphy (1967) are based upon the competition for binding sites between steroids extracted from the blood and a radioactive labelled hormone added to the test system. Corticoid and progesterone assays utilize corticosteroid binding globulin of dog plasma and the estrogen assay utilized rabbit uterine cytosol as the binding protein. Excess unbound steroids are removed by an absorbent and the labelled steroid remaining in the test system are counted to quantitate the competitive reaction. The larger the amount of labelled steroid remaining bound to the protein, the smaller the amount of unlabelled steroid in the test system.

Steroid standards (cortisol, progesterone, estrone and estradiol-17 $\beta$ ) were purchased from Calbiochem<sup>1</sup>. Isotope

---

<sup>1</sup>Calbiochem., La Jolla, Calif.

labelled steroids were purchased from New England Nuclear Corporation.

## 2. Corticosteroids

Plasma samples were assayed for total corticoid content utilizing the protein binding method of Murphy (1967) as modified by Whipp and Lyon (1970). Details of the test procedure are outlined in Appendix Table 1.

Plasma samples were extracted with methylene chloride and paired one ml aliquots dried. Each sample was quantified in a protein binding system utilizing 3% dog corticoid binding globulin and  $^3\text{H}$ -cortisol. Tritium activity was determined in a Beckman LS-100 scintillation counter. Corticoid content in each sample was determined from a regression equation formulated from the corticoid standards.

## 3. Progesterone

Plasma samples were assayed for progesterone using a protein binding method described by Neil et al. (1967) with slight modifications. Details of the test procedure are outlined in Appendix Table 2.

The method consists of extraction with petroleum ether, purification by thin-layer chromatography and quantification in a protein binding system consisting of dog corticoid binding globulin (3% CBG) and  $^3\text{H}$ -corticosterone. Tritium activity was determined in a Beckman LS-100 scintillation counter.

Progesterone concentration of each sample was determined from  $^3\text{H}$  activity compared to progesterone standards and correction for procedural losses. The correction factor was determined as the percentage of  $^{14}\text{C}$ -progesterone remaining in the sample at the end of the test procedure.

#### 4. Estrogens

Plasma samples were assayed for estrone and estradiol using the radio-ligand assay technique described by Korenman, Tulchinsky and Eaton (1970). Details of the test procedure are outlined in Appendix Table 3.

Plasma samples were extracted twice with freshly opened anhydrous diethyl ether. The extracts were dried and placed on celite columns. Elution solvents used were iso-octane (trimethyl pentane), 15% ethylacetate in iso-octane and 30% ethyl acetate in iso-octane. Estrone ( $\text{E}_1$ ) was obtained in the 15% ethyl acetate fraction and estradiol ( $\text{E}_2$ ) in the 30% ethylacetate fraction. The eluates were dried, reconstituted in tris-HCL buffer and placed in duplicate 12 x 75 mm tubes. The protein used was rabbit uterine cytosol. Samples were incubated for 24 hours at 4 C, and then separated using dextran coated charcoal. Supernatant radioactivity was determined in a Beckman LS-100 counter.

## C. Statistical Analysis

Maternal plasma steroid levels were analyzed using a least-squares analysis of variance program written by Harvey. The procedure for analysis of unequal sub-class numbers is described by Kempthorne (1952) and Harvey (1960).

The following experimental models were used:

$$Y_{ijk} = \mu + t_i + s_j + e_{ijk}$$

where  $Y_{ijk}$  = maternal progesterone in DXMS treated cows

$\mu$  = sample mean

$t_i$  = sample time

$t_1$  = 24 hours before DXMS

$t_2$  = 24 hours after DXMS

$t_3$  = 24-48 hours after DXMS

$s_j$  = sample site

$s_1$  = jugular veins

$s_2$  = uterine vein

$s_3$  = utero-ovarian veins

$$Y_{ijk} = \mu + t_i + s_j + e_{ijk}$$

where  $Y_{ijk}$  = maternal estrone in DXMS treated cows

$\mu$  = sample mean

$t_i$  = sample time ( $t_1, t_2, t_3$ )

$s_j$  = sample site  $s_1$  and  $s_3$

$$Y_{ijk} = \mu + t_i + s_j + e_{ijk}$$

where  $Y_{ijk}$  = maternal corticoids in DXMS treated cows

$\mu$  = sample mean

$t_i$  = sample time ( $t_1$ ,  $t_2$ , and  $t_3$ )

$s_j$  = sample site ( $s_1$ ,  $s_2$ , and  $s_3$ )

$$Y_{ijk} = \mu + t_i + c_j + e_{ijk}$$

where  $Y_{ijk}$  = maternal progesterone in HCG and DXMS treated cows

$\mu$  = sample mean

$t_i$  = sample time ( $t_1$ ,  $t_2$ , and  $t_3$ )

$c_j$  = calving status ( $c_1$  = calve,  $c_2$  = not calve)

$$Y_{ijk} = \mu + t_i + c_j + e_{ijk}$$

where  $Y_{ijk}$  = maternal estrone in HCG and DXMS treated cows

$\mu$  = sample mean

$t_i$  = sample time ( $t_1$ ,  $t_2$ , and  $t_3$ )

$c_j$  = calving status ( $c_1$  or  $c_2$ )

$$Y_{ij} = \mu + t_i + e_{ij}$$

where  $Y_{ij}$  = maternal progesterone in HCG and DXMS treated cows that calved (group 1) or not calving (group 2)

$\mu$  = sample mean

$t_i$  = sample time ( $t_1$ ,  $t_2$ , and  $t_3$ )



$$Y_{ij} = \mu + t_i + e_{ij}$$

where  $Y_{ijk}$  = maternal estrone in HCG and DXMS treated cows  
that calved (group 1) or not calving (group 2)

$\mu$  = sample mean

$t_i$  = sample time ( $t_1$ ,  $t_2$ , and  $t_3$ )

## IV. RESULTS

## A. Postpartum Reproductive Involution

1. Gross observations

Uterine involution was evaluated grossly and histologically at two, four and six weeks postpartum. The gross observations of reproductive and endocrine glands at slaughter are listed in Table 1. Rectal evaluations of uterine involution and ovarian function of 23 cows are listed in Table 2.

The corpus luteum of the prior pregnancy was hardly discernible via rectal palpation. At necropsy, the average diameter of the corpus luteum was 1.0, 0.8 and 0.8 cm at two, four and six weeks postpartum, respectively.

The uterine horn of prior pregnancy averaged 9.8 cm in diameter at the bifurcation and 5.6 cm in diameter across the non-gravid horn when slaughtered at two weeks. At two weeks postpartum, rectal palpation of 23 cows revealed an average uterine horn size of 8.3 and 4.7 cm for cows which had retained placentae. Cows without placental retention averaged 6.8 and 3.8 cm across the pregnant and non-pregnant uterine horns, respectively, at the same postpartum interval.

There was little difference in uterine size between cows examined rectally or at slaughter or between normal cows and those with prior placental retention when examined at four and six weeks postpartum (Table 1 and Table 2). Four weeks after

Table 1. Post mortem evaluation of reproductive involution<sup>a</sup>

Interval (weeks)	2	4	6
No. of cows	2	3	3
Diameter CL of preg. (cm)	1	0.8	0.8
No. cyclic CLs	0	1	1
No. of Follicles over 1 cm	3	3	2
Cows in estrus prior to slaughter	0	1	1
Uterine wt. gravid horn (g)	725	245	275
Uterine wt. non-gravid horn (g)	288	108	170
Uterine size gravid horn (cm)	9.8	3.9	3.1
Uterine size non-gravid horn (cm)	5.6	2.2	2.5
Uterine lochia (ml)	460	30	0
Left adrenal wt. (g)	17.6	14.0	20.7
Right adrenal wt. (g)	15.3	12.6	18.3
Thyroid wt. (g)	30.8	30.9	35.3
Pituitary wt. (g)	2.7	2.4	3.4
Ant. pit. wt. (g)	2.4	2.2	2.9

<sup>a</sup>Mean values for each group.

Table 2. Ante mortem evaluation of reproductive involution

Postpartum interval (weeks)	2	4	6
Cows in estrus or with cyclic Cl n(23)	0	3	7
Cows with placental retention n(16)			
Gravid horn size (cm)	8.3	4.0	3.2
Non-gravid horn size (cm)	4.7	3.1	2.8
Cows without placental retention n(7)			
Gravid horn size (cm)	6.8	3.8	3.0
Non-gravid horn size (cm)	3.8	2.5	2.6

calving the uterine horn of pregnancy was nearly 3.8 cm in diameter and the other horn nearly 2.5 cm. At six weeks the prior gravid and non-gravid horns were nearly 3.1 and 2.7 cm, respectively.

Uterine weight decreased rapidly from 1013 g at two weeks to 353 g at four weeks postpartum. Uterine weight at six weeks averaged 445 grams. There was a progressive decrease in uterine lochia from 460 ml at two weeks to about 30 ml at four weeks and none at six weeks.

None of the cows showed evidence of estrus or ovulation by two weeks postpartum, although two cows at slaughter had one or more follicles over 1 cm in diameter. Four weeks after calving, one of three slaughtered cows and three of the 23

palpated cows showed evidence of ovulation or estrus. Two of three slaughtered cows and seven of 23 palpated cows were in estrus or had ovulated by six weeks after calving.

## 2. Histological observations in normal cows

Uterine and ovarian tissues from the induced cows were examined for histopathological variation from the normal histology of the postpartum cows. Histological sections from normal nursed cows collected at 7, 14, 30 and 42 days postpartum served as controls. The histological involution of the uterus and ovaries of the normal cows are described below.

The caruncular surface was devoid of epithelium in seven day postpartum cows. A definite line of demarcation divided the vital tissue of the caruncle and the surface tissue destined to be sloughed (Figure 1). Histiocytic cells with foamy cytoplasm, scant hemosiderin pigments, and vacuoles were the primary cells in the necrotic area. Dispersed within the mass were large, often binucleated, trophoblastic cells of the chorionic villi which showed coagulative necrosis. The chorionic villi were fibrinoid in appearance. A few cryptal giant cells were present at the base of the endometrial crypts particularly near the degenerating placental villi (Figure 2). Some of the caruncular surface had sloughed. Only a few foci of polymorphonuclear cells were evident in the caruncular area, though they were more numerous near the surface.

Beneath this necrotic mass, the lamina propria was composed of a fibrous connective tissue meshwork with capillaries, and a few large arterioles extending from the basal stalk to near the line of demarcation. These arterioles had thickened walls and narrowed lumens (Figure 3). Endothelial cells protruded into the lumens. Thrombosed arterioles extended up into the necrotic caruncular surface. Histiocytes, fibroblasts, a few lymphocytes and occasional foci of polymorphonuclear cells were found within the lamina propria.

The intercaruncular surface epithelium showed considerable variation. It consisted of columnar epithelium with occasional areas of focal necrosis. Some cells had large vacuoles near the basement membrane. Few neutrophils were present in areas of intact epithelium, but extensive polymorphonuclear cell infiltration was common in those uteri which had placental remnants. Simple non-branching and endometrial glands with small lumens were usually present. The epithelia of glands occasionally showed mitotic figures and piling of nuclei near the endometrial crypts. These glands had distended lumens when intercaruncular necrosis was extensive.

At this stage of involution the myometrium comprised approximately 2/3 of the (12-15 mm thick) uterine wall. Larger arteries appeared thickened and tortuous but no degenerative changes were noted in these vessels.

In the 14 day postpartum cow the caruncular surface was fragmented with bits of necrotic tissue, though most of the devitalized tissue had sloughed and no line of demarcation was evident (Figure 5). The caruncular surface was composed mostly of distended histiocytes and foci of neutrophils. Fibroblasts and lymphocytes were numerous in the basal portion of the caruncule. A moderate number of plasma cells was also present.

Placental remnants appeared as an eosinophilic mass with pyknotic nuclei. A few cryptal giant cells and many histiocytes surrounded the placental remnants and multiple foci of neutrophils were seen.

The caruncular arteries and arterioles showed some degree of hyaline degeneration and reduced lumen size (Figure 6). Those most proximal to the caruncular surface were reduced in lumen diameter mainly by connective tissue replacement of the media. A few of the larger arteries in the septal mass and adjacent myometrium showed hyaline degeneration of the media. The media appeared as an amorphous eosinophilic mass with few nuclei. Small foci of lymphocytes occasionally were seen near these vessels. There was distention of the venules in the caruncular area. The adventitia of some veins were thickened. These vessels showed increased eosinophilia, waviness and clefts in the adventitia.

The intercaruncular epithelium was simple columnar to cuboidal with a few vacuoles and hyaline droplets (Figure 7).

More severely involved epithelium showed larger vacuoles and granular cytoplasm. Neutrophils were numerous within the submucosa and immediately beneath the epithelium. The endometrial stroma contained many histiocytes, particularly in the subepithelial area. Fibroblasts and lymphocytes were more numerous in the deeper lamina propria. Plasma cells were evident as small perivascular foci or were scattered within the lamina propria. Some uteri had pseudostratified epithelium which projected into the uterine lumen as small hyperplastic villi.

The deeper lying submucosa was more collagenous and less vascular than the upper layer. Endometrial glands were scant in some uteri and more numerous in other uteri regardless of the degree of inflammation in the upper layers.

The myometrium was approximately three times as thick as the endometrium. The myometrium being about 8-10 mm thick, was thrown into longitudinal folds. Larger vessels within the myometrium were thick and tortuous. Low cuboidal mesothelial epithelium covered the serosal surface.

In the 28 day postpartum uteri, the caruncle was covered by low columnar epithelium peripherally to a low cuboidal and barely discernible cellular layer over the center (Figure 8). Below this delicate epithelial cover, the stratum compactum appeared as chronic inflammation with histiocytes, fibroblasts, and multiple lymphocytic foci near the surface. Many of the



occluded arterioles near the surface were replaced by connective tissue. A few of the deeper septal arterioles and arteries showed evidence of re-channeling of occluded lumens, with much of the intimal and medial layers replaced with fibrous connective tissue (Figure 9).

The intercaruncular epithelium was simple columnar (Figure 10). Histiocytes, capillaries and foci of lymphocytes comprised most of the stratum compactum. Endometrial glands coursed through this zone. The deeper lamina propria was composed of a less vascular tissue which had many branched and coiled endometrial glands in a loosely arranged fibrous connective tissue zone. The uterine wall, averaged about 10 mm in thickness, and had not changed appreciably in thickness when compared to a 14 day postpartum uterus. However, two distinct differences could be noted. The endometrial thickness was approximately doubled in the intercaruncular area, due primarily to more endometrial glands and more connective tissue support in the deeper lamina propria. This represented a change from 1.5 to 2 mm to approximately 3 or 4 mm in thickness.

Another distinct difference between the 14 day and 28 day postpartum uteri was a 1/3 reduction in the myometrial thickness. This reduction in myometrium was due to muscle atrophy. Smooth muscle cell nuclei were tightly packed due to general atrophy of the cytoplasm. A few muscle cells appeared to have small darker staining nuclei with hyaline staining cytoplasm.

Microscopic examination of the 42 day postpartum uteri revealed intact simple columnar epithelium covering the caruncle (Figure 11). The caruncles had regressed in size to approximately 0.4 to 1.8 cm. Lymphocytic foci were often scattered through the stratum compactum which still had a few reactive histiocytes. Pseudostratified columnar epithelium covered the intercaruncular surface. Capillaries and supporting fibroblasts, with a few lymphoid follicles, comprised most of the upper lamina propria. The deeper lamina propria was less vascular with loosely arranged connective tissue support and many branching endometrial glands. The endometrium was 2 to 3 mm thick.

The myometrium was approximately 4 to 6 mm thick. It was composed of densely packed smooth muscle cells. The nuclei of most of these muscle cells touched or overlapped when examined from a longitudinal section. The area between the circular and outer longitudinal smooth muscles had many large arteries and veins. These vessels were very tortuous and thick with considerable connective tissue around them. The endothelium of many of these arteries were thrown into irregular longitudinal folds. Some vessels showed increased sub-endothelial connective tissue.

Deep longitudinal folds and rugae were characteristic of serosal surface. It was covered by cuboidal mesothelium.

The ovarian histology of seven days postpartum cows revealed multiple immature follicles. The largest follicles had atria about 7 mm in diameter. These follicles showed atretic changes consisting primarily of degenerative changes in the theca and granulosa cells. The cytoplasm of such cells were very eosinophilic and pyknotic nuclei were frequently observed. The theca interna cells showed granular clumping in their cytoplasm and many fibroblasts among them. Peripherally the theca externa was indistinguishable from the surrounding ovarian stroma.

The corpus luteum of pregnancy, while still quite evident, had regressed to approximately 12 mm in diameter. Severe signs of degeneration including nuclear pyknosis and vacuolation of the cytoplasm were observed in the luteal cells (Figure 12). Most luteal cells had scant cytoplasm, but some cells were distended with hyaline staining cytoplasm and multiple small vacuoles. Thick arterioles and fibroblastic cells comprised approximately half of the corpus luteum.

In 14 day postpartum cows, the corpora lutea of pregnancy were 7 to 9 cm in diameter. They were composed of luteal cells separated by fibrous trabeculae and many arteries and veins (Figure 13). A few cells were seen laden with light brown pigment. The nuclei were small and darkly staining and often eccentrically positioned. It was difficult to identify these cells as either luteal or histiocytic, but they were observed

in degenerative corpora lutea long after the luteal cells cease to function. Vascular tissue comprised about 3/4 of the luteal mass. Larger vessels had thickened walls and nearly occluded lumens.

Some 14 day ovaries had nearly mature Graafian follicles with a distinct 15-20 follicular cell layer. The theca interna cells of these follicles were laden with lipid-like foamy pink cytoplasm. A few follicles which had failed to mature had mulberry shaped, pink staining, Call-Exner bodies (Figure 14).

At 28 days postpartum, the corpus luteum of pregnancy was nearly replaced by fibrous connective tissue. Most of the vasculature of the old corpus luteum had also been replaced by fibrous connective tissue (Figure 15). Both atretic follicles with Call-Exner bodies and nearly mature follicles were seen.

### 3. Histological observations in DXMS treated cows

Histologically the uterine involution two weeks after DXMS induced parturition was most characteristic of the normal 14 day postpartum cow, but the number of polymorphonuclear cells and the degree of intercaruncular epithelial necrosis was more characteristic of the normal seven day postpartum uterus.

Most of the non-vital tissue of the caruncle had detached by 14 days. Fragments of necrotic tissue, usually containing fetal membrane remnants, covered the caruncular surface.

Polymorphonuclear cells were exceedingly numerous within the uterine lumen. They were equally numerous on the surface and within the caruncular mass. Multinuclear giant cells were occasionally seen. Histiocytes, lymphocytes and plasma cells appeared in a frequency consistent with the normal 14 day postpartum uterus. Vascular contraction and thrombosis within the septal mass was equal to what was observed in the normal cow.

The intercaruncular endometrium was often devoid of surface epithelium with extensive polymorphonuclear cell infiltration when acute inflammation followed retention of the fetal membrane (Figure 16).

In other cows the intercaruncular epithelium was made up of columnar cells which contained vacuoles and hyaline droplets. The lamina propria contained polymorphonuclear cells and histiocytes in numbers equivalent to the normal 14 day postpartum cows. Plasma cells and lymphocytes were proportionately evident. Endometrial glands were scant and often had distended lumens.

A few uteri had less reactive cells but more edema in the endometrium and myometrium. Myometrial cells from such uteri were large with hyaline appearing cytoplasm (Figure 17).

Uterine involution at 28 days postpartum in induced cows was slightly delayed histologically compared to the normal 28 day cows. The caruncular surface was usually covered with low cuboidal epithelium, but a few cows with prior placental

retention did not have complete epithelial covering of the caruncle. Plasma cells and foci of lymphocytes were more numerous in the caruncles of induced cows. Caruncular size and vascular changes were comparable with the normal cows.

The intercaruncular epithelium was simple columnar. The endometrium contained more polymorphonuclear cells, plasma cells and lymphocytes in induced cows (Figure 18). Endometrial gland hyperplasia occurred as frequently as seen in the normal cows.

Involution of the myometrium, as evidenced by vascular contraction and myometrial cell atrophy was indistinguishable from the normal cows at four weeks postpartum.

Histologically, uterine involution in the 42 day postpartum induced cows was equal to the normal cow in nearly all respects. A slightly higher frequency of lymphoid follicles within the stratum compactum of the endometrium was evident in some sections. Endometrial gland hyperplasia was present in nearly all sections.

#### 4. Histological comparison of normal and DXMS treated cows

Regression of the corpus luteum of pregnancy and the return of follicular activity in the ovaries of induced cows was equal to that observed in the normal cows at postpartum.

In order to be more objective the uterine tissue sections were observed for the following individual tissue changes:

Figure 1. Photomicrograph of caruncle from seven day postpartum cow

Upper left. Normal cow, day 7 postpartum. Cross-section of caruncle. The line marking the limit of necrosis and sloughing of the caruncular surface is very prominent (D) (H & E, X40)

Figure 2. Photomicrograph of cryptal giant cells

Upper right. Normal cow, day 7 postpartum. Caruncular tissue with cryptal giant cells (G) in area destined to be sloughed (H & E, X400)

Figure 3. Photomicrograph of caruncular arteriole from seven day postpartum cow

Lower left. Normal cow, day 7 postpartum. Caruncular arteriole with narrowed lumen and endothelial cells protruding into the lumen (H & E, X100)

Figure 4. Photomicrograph of intercaruncular endometrium from seven day postpartum cow

Lower right. Normal cow, day 7 postpartum. Normal intercaruncular epithelium and lochia in the lumen of the uterus (H & E, X100)

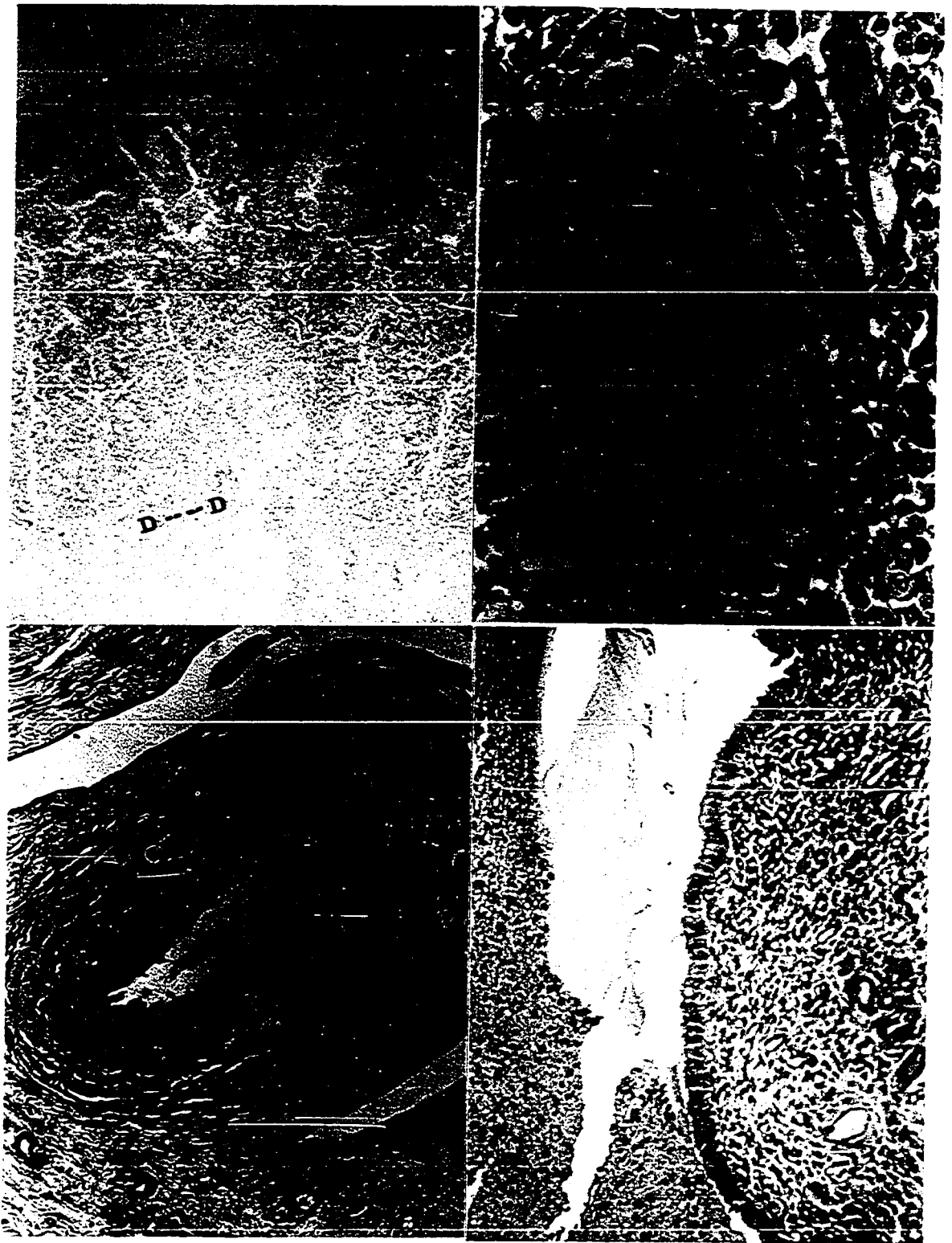




Figure 5. Photomicrograph of caruncle from 14 day postpartum cow

Upper left. Normal cow, day 14 postpartum. Cross-section of caruncular surface after necrotic tissue has been sloughed (H & E, X100)

Figure 6. Photomicrograph of septal artery from 14 day postpartum cow

Upper right. Normal cow, day 14 postpartum. Septal artery with hyaline degeneration of the media (A) (H & E, X100)

Figure 7. Photomicrograph of intercaruncular endometrium from 14 day postpartum cow

Lower left. Normal cow, day 14 postpartum. Intercaruncular epithelium with large cytoplasmic vacuoles, hyaline droplets (H) and neutrophils (H & E, X400)

Figure 8. Photomicrograph of caruncle from 28 day postpartum cow

Lower right. Normal cow, day 28 postpartum. Cross-section of caruncular surface covered by low cuboidal epithelium. Lymphocytic foci are evident in the stratum compactum (L) (H & E, X100)

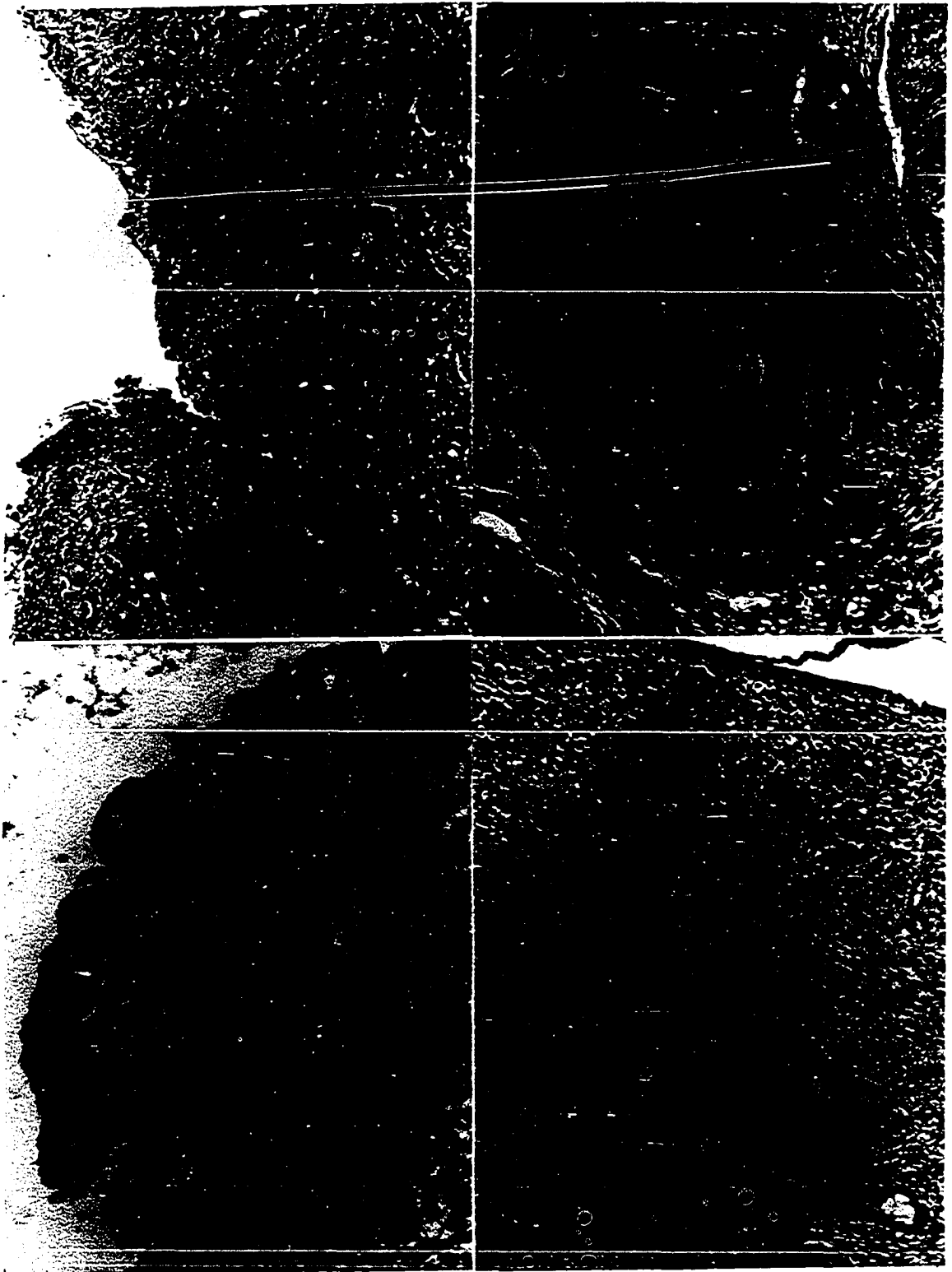


Figure 9. Photomicrograph of septal artery from 28 day postpartum cow

Upper left. Normal cow, day 28 postpartum. Septal artery with re-channeling of an occluded lumen (O) (H & E, X100)

Figure 10. Photomicrograph of intercaruncular endometrium from 28 day postpartum cow

Upper right. Normal cow, day 28 postpartum. Intercaruncular epithelium of simple columnar cells. Endometrial glands are numerous in the lamina propria (H & E, X40)

Figure 11. Photomicrograph of caruncle from 42 day postpartum cow

Lower left. Normal cow, day 42 postpartum. Cross-section of a caruncle, with intact simple columnar epithelium, and arterioles (A) with thick walls and small lumens (H & E, X40)

Figure 12. Photomicrograph of corpus luteum of pregnancy from seven day postpartum cow

Lower right. Normal cow, day 7 postpartum. Section of corpus luteum of pregnancy with pyknotic nuclei and cytoplasmic vacuolation. A few luteal cells were distended with hyaline staining cytoplasm (B) (H & E, X100)

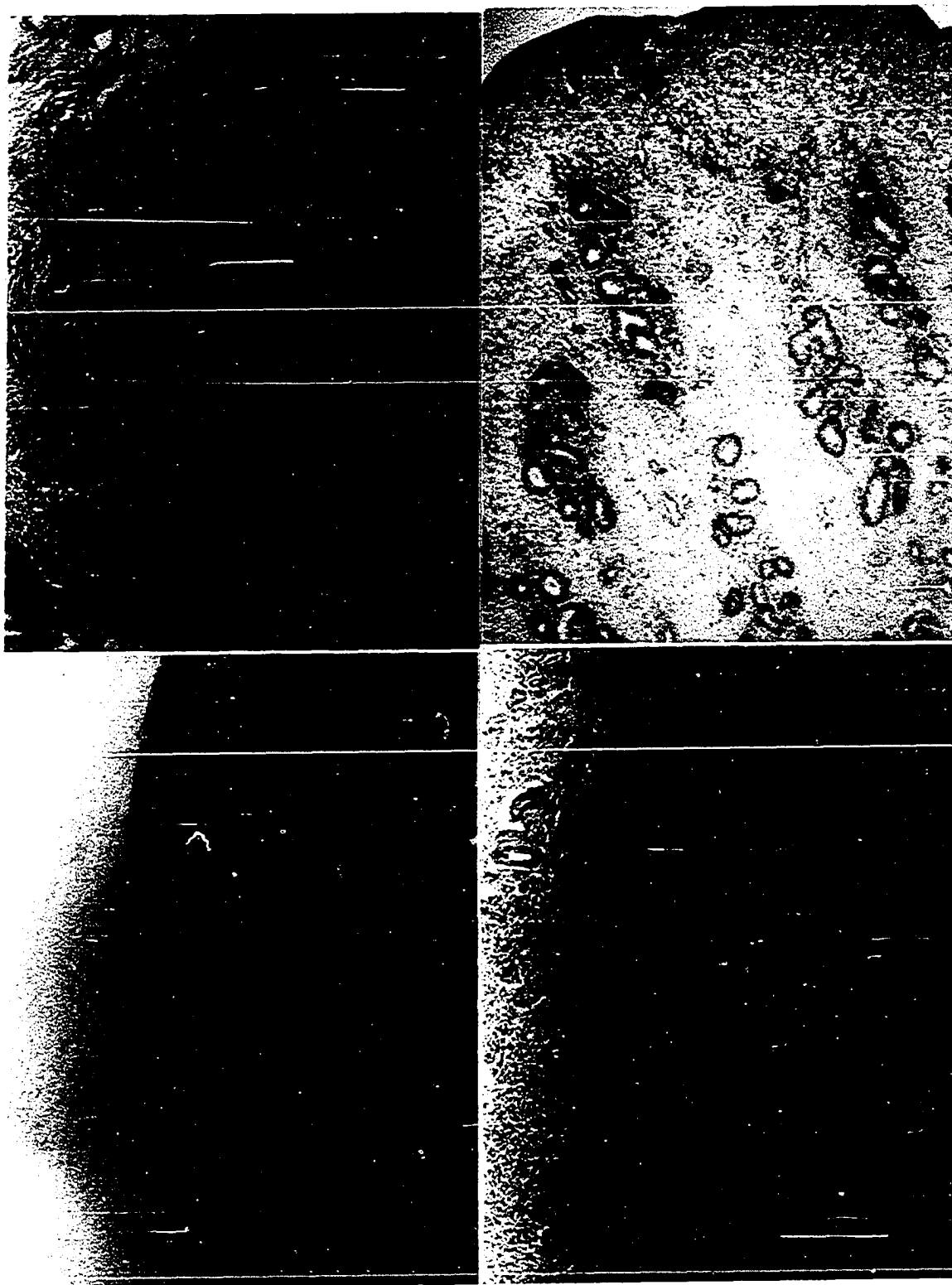


Figure 13. Photomicrograph of corpus luteum of pregnancy from 14 day postpartum cow

Upper. Normal cow, day 14 postpartum. Section of corpus luteum of pregnancy. Many thick walled arteries (A) and small luteal cells are evident.

Figure 14. Photomicrograph of Call-Exner bodies

Lower left. Normal cow, day 14 postpartum. Section of an atretic follicle wall. Palisade of granulosa cells line the wall. Call-Exner bodies (B) with eosinophilic centers were evident (H & E, X400)

Figure 15. Photomicrograph of corpus luteum of pregnancy from 28 day postpartum cow

Lower right. Normal cow, day 28 postpartum. Section of corpus luteum of pregnancy which was composed mostly of large and small arteries, and fibrous connective tissue (H & E, X100)

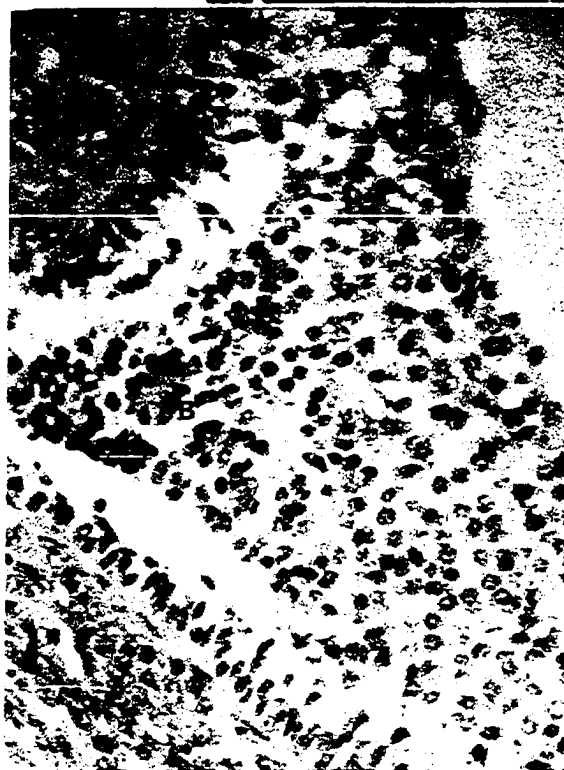
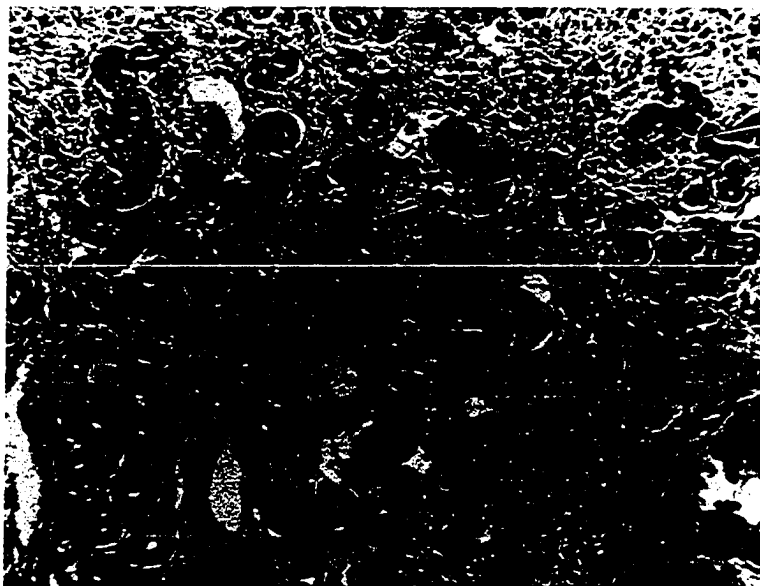


Figure 16. Photomicrograph of intercaruncular endometrium from 14 day postpartum DXMS treated cow

Upper. DXMS cow, day 14 postpartum. Intercaruncular endometrium was devoid of surface epithelium. Both the surface and lamina propria was filled with polymorphonuclear cells (H & E, X40)

Figure 17. Photomicrograph of myometrium from 14 day postpartum DXMS treated cow

Lower left. DXMS cow, day 14 postpartum. Section of myometrium in cow with retained placenta. Myometrium was lacy in appearance and the nuclei sparsely spaced. Hyaline staining cytoplasm (A) was common in the myometrial cells (H & E, X400)

Figure 18. Photomicrograph of intercaruncular endometrium from 28 day postpartum DXMS treated cow

Lower right. DXMS cow, day 28 postpartum. Section of intercaruncular endometrium with lochia on the surface. Polymorphonuclear cells were numerous in the surface and lamina propria. Lymphocytes and plasma cells were also numerous in the lamina propria (H & E, X100)





regeneration of the caruncular epithelium, caruncular size and resolution, regeneration of intercaruncular epithelium, endometrial gland activity, neutrophil infiltration, myometrial involution and vascular changes within the caruncle and the myometrium. Each characteristic was rated from one to four respectively according to their similarity to the 7, 14, 28 or 42 day normal postpartum uteri. A mean rating was determined for each characteristic at two, four and six weeks after induced parturition. Induced cows with placental retention were compared graphically with induced cows which did not have placental retention (Figure 19).



Epithelization of the caruncle, caruncular resolution and vascular resolution progressed in nearly equal rates in the two groups. Both groups also compared favorably with the normal cows in these categories.

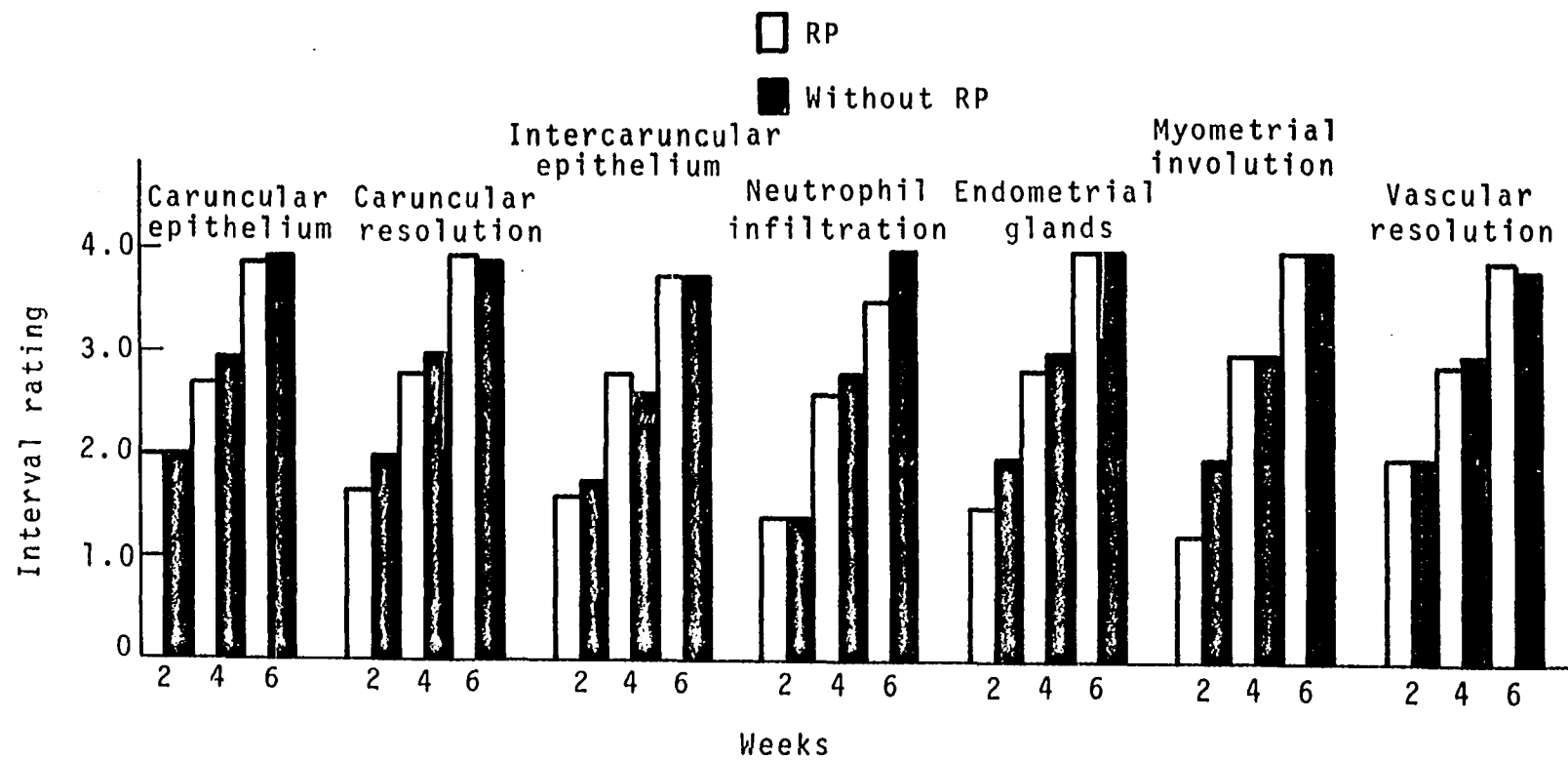
Neutrophil infiltration was higher in both groups of DXMS treated cows at two weeks and remained more numerous in the retained placenta cows at four and six weeks postpartum.

Endometrial gland resolution and proliferation was slightly delayed in retained placenta cows, but by four and six weeks both groups rated closely to the normal postpartum cows which did not receive DXMS.

Placental retention caused a delay in myometrial involution primarily as delayed contraction and increased myometrial edema at two weeks postpartum. Uteri examined at four and six

Figure 19. Graphic illustration of histological evaluation of uterine involution in DXMS treated cows

Graphic illustration comparing histological evaluation of uterine involution in DXMS treated cows with  retained placentas and without  retained placentas. Interval rating of 1, 2, 3 or 4 is based upon similarity to normal postpartum cows at 7, 14, 28 and 42 days respectively.



weeks were equal to myometrial involution and were rated equal to normal cows.

Both groups of DXMS treated cows showed vacuolation and regressive changes in the intercaruncular epithelium at two weeks postpartum. Some sections which had more severe inflammation had focal areas of necrosis. Both groups were assessed to have nearly normal epithelium at four and six weeks postpartum.

#### B. Steroid Levels in DXMS Treated Cows

##### 1. Corticosteroids

The corticoid concentrations in jugular plasma of three DXMS treated cows are shown in Figure 20. Presurgical corticoids averaged near 8 ng/ml. Samples obtained immediately after surgery were consistently higher, averaging 14.5 ng/ml. Corticoid levels fell below presurgical levels following DXMS treatment, until just prior to parturition when a second peak of 11 ng/ml occurred.

Cow 20, which calved without treatment, averaged 8.4 ng corticoids/ml plasma for five days prepartum (Figure 21) and then rose to 24 ng/ml at calving.

Plasma samples collected from three sample sites in cows 22 and 23 were assayed for corticoid content (Figure 22). Sites of blood collection were the jugular vein, uterine vein and utero-ovarian vein. DXMS treatment depressed plasma

Figure 20. Jugular plasma corticoids (ng/ml, mean + SE) in DXMS treated cows (n = 3) before and after parturition

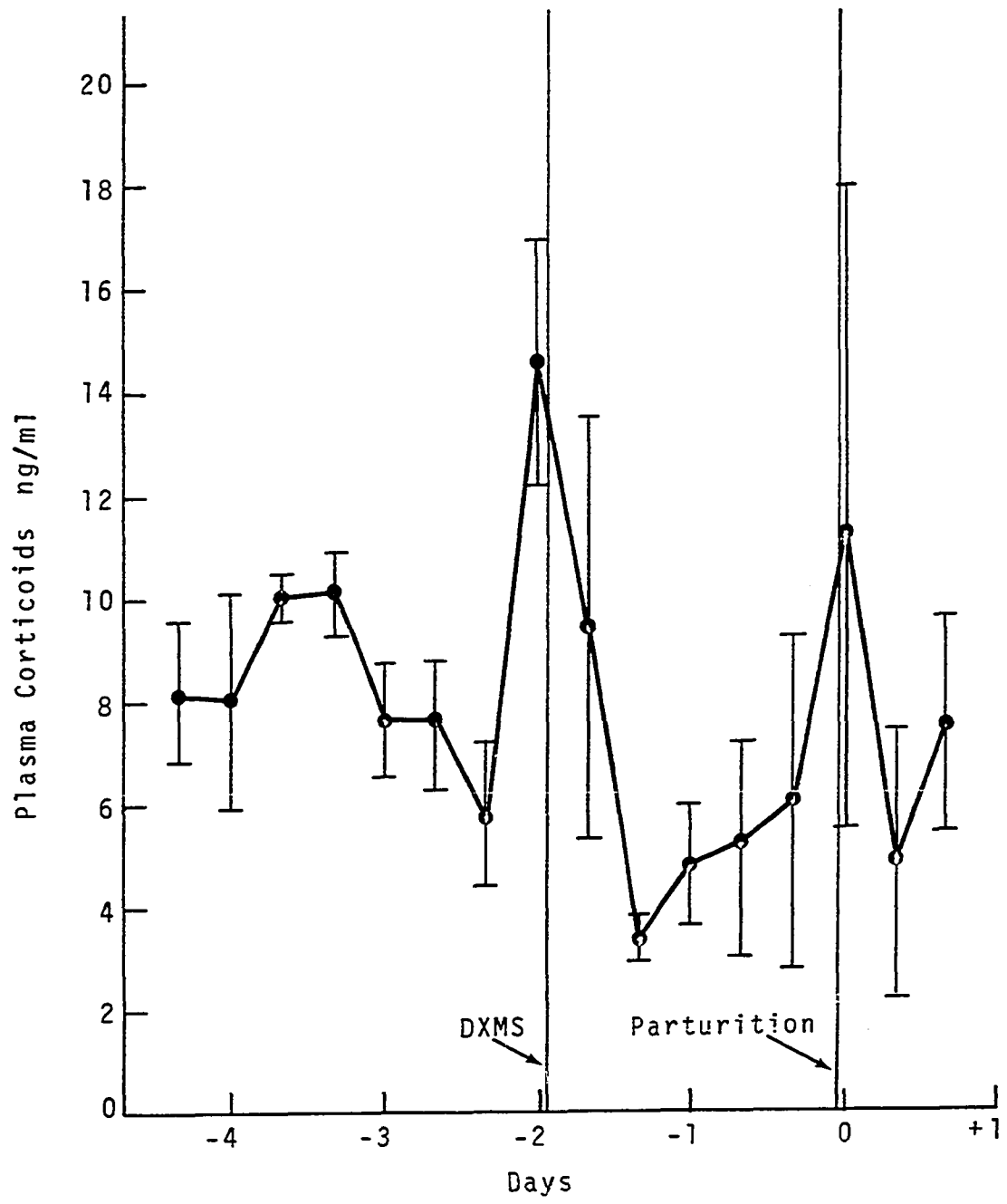


Figure 21. Jugular plasma corticoids (ng/ml) in naturally calving cow 20

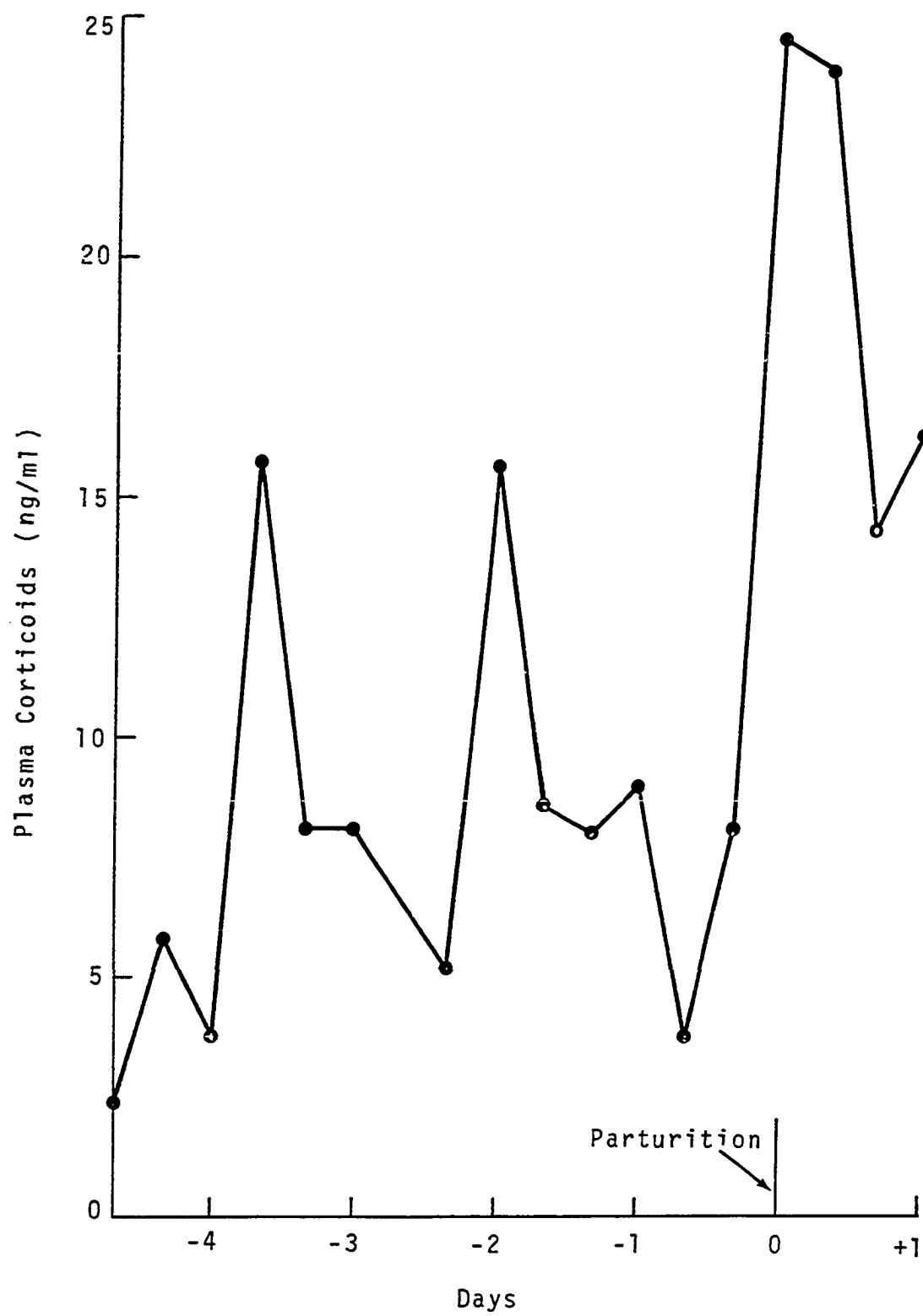
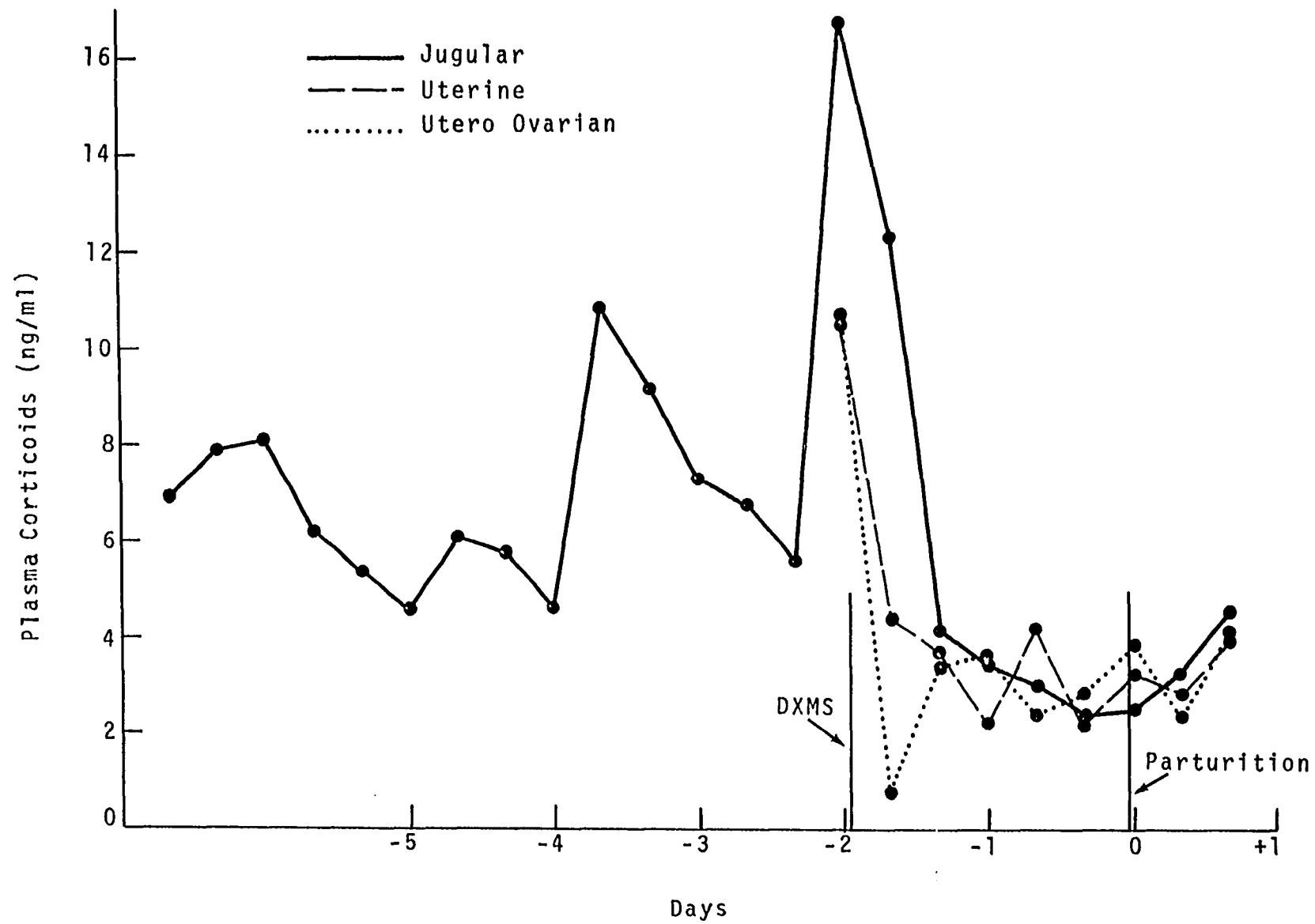




Figure 22. Plasma corticoids (ng/ml) in jugular, uterine and utero-ovarian veins of DXMS treated cows (n = 2)

Uterine and utero-ovarian samples were taken before and after DXMS treatment.



corticoids at all sample sites. A prepartum increase was absent in these two cows. These sample averages were tested statistically for significant differences in corticoid content between sample sites or between time of collection (Table 3).

Utilizing least squares analysis of variance, a significant difference ( $P < .05$ ) was found between pretreatment and post-treatment plasma corticoid levels. No significant difference occurred between sample sites, nor was there any significant interaction between time and sample sites.

Table 3. Plasma corticoids (ng/ml, mean  $\pm$  SE) in DXMS treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
	8.81 <sup>b</sup>	4.13	2.98 $\pm$ 0.36
Site <sup>c</sup>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>
	6.31 $\pm$ 1.12	4.19 $\pm$ 0.90	5.43 $\pm$ 1.11

<sup>a</sup>T<sub>1</sub> = 24 hours prior to DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24-48 hours after DXMS.

<sup>b</sup>T<sub>1</sub> significantly different ( $P < .05$ ) from T<sub>2</sub> and T<sub>3</sub>.

<sup>c</sup>S<sub>1</sub> = jugular v. S<sub>2</sub> = uterine v. S<sub>3</sub> = utero-ovarian v.

## 2. Progesterone

Jugular plasma progesterone levels averaged 5.8 ng/ml for five days preceding dexamethasone treatment (Figure 23) in cows 1, 2, 22, 23 and 24. Post treatment progesterone levels declined rapidly from 7.3 to 1.6 ng/ml the first 24 hours and continued to fall to less than 1 ng/ml until calving commenced less than 48 hours after treatment. Jugular plasma progesterone in the control cow (no. 20) followed a similar pattern of decline prior to calving (Figure 23). Progesterone levels averaged nearly 4.2 ng/ml for five days prior to a precipitous fall about 48 hours prior to calving. Plasma progesterone averaged 0.7 ng/ml for the 32 hours preceding calving.

Plasma progesterone averaged about 6 ng/ml prior to DXMS treatment in cow 38 (Figure 23). Post treatment levels averaged 4.6 ng/ml for 72 hours and parturition did not occur until 12 days after DXMS administration.

Plasma samples collected hourly from three sites following surgery were assayed for progesterone content (Figure 24) in cows 1, 2, 22, and 23. Samples collected immediately after surgery averaged 6.5, 7.3, and 22.8 ng/ml respectively from the uterine, jugular and utero-ovarian veins. Utero-ovarian levels were three to four times greater than the levels at other sample sites for nearly 15 hours after DXMS treatment.

Uterine vein progesterone content was consistently below jugular vein levels. Progesterone content declined slowly in

Figure 23. Jugular plasma progesterone (ng/ml) in DXMS treated cows (C) calving, (NC) not calving, and in naturally calving cow

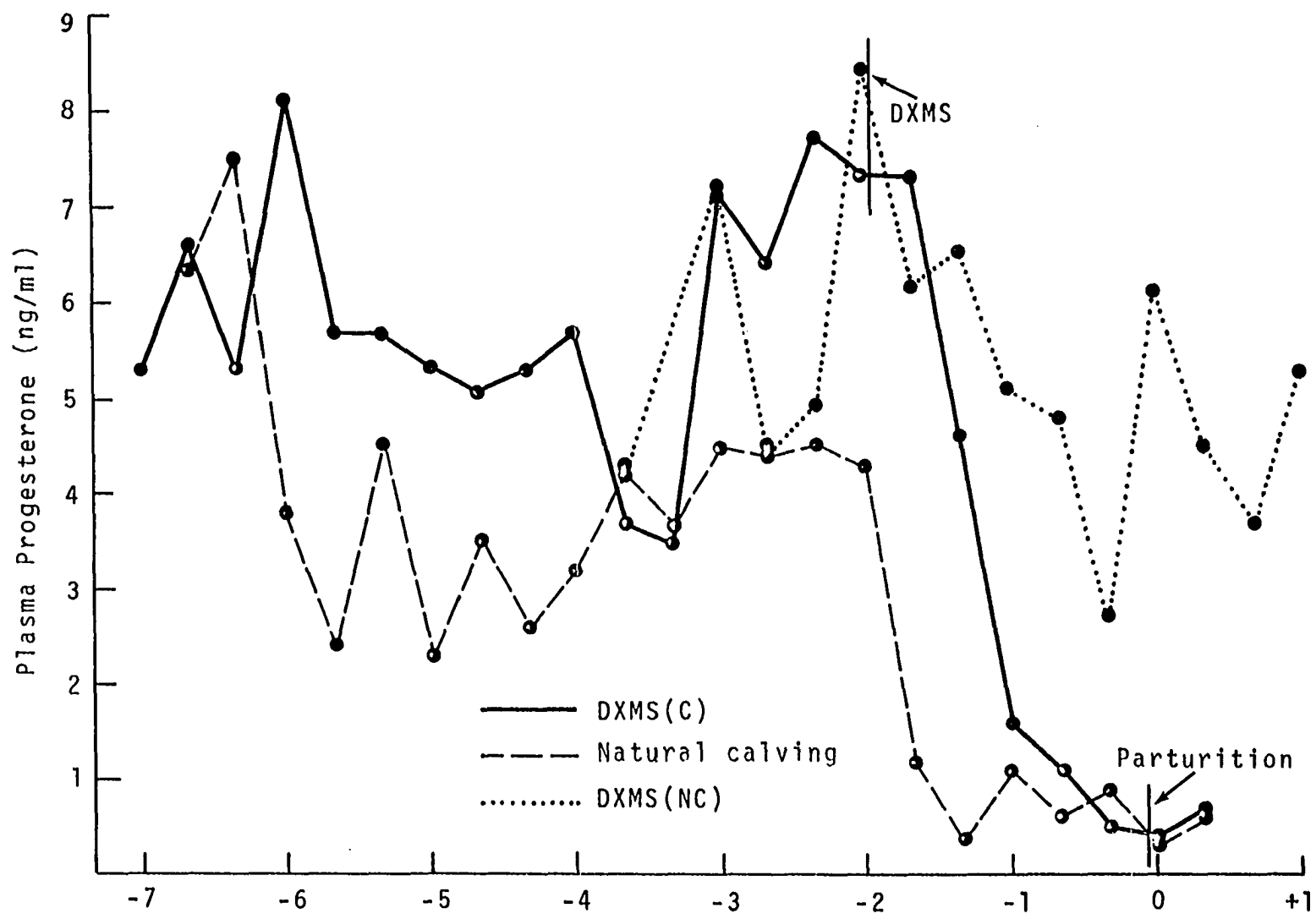
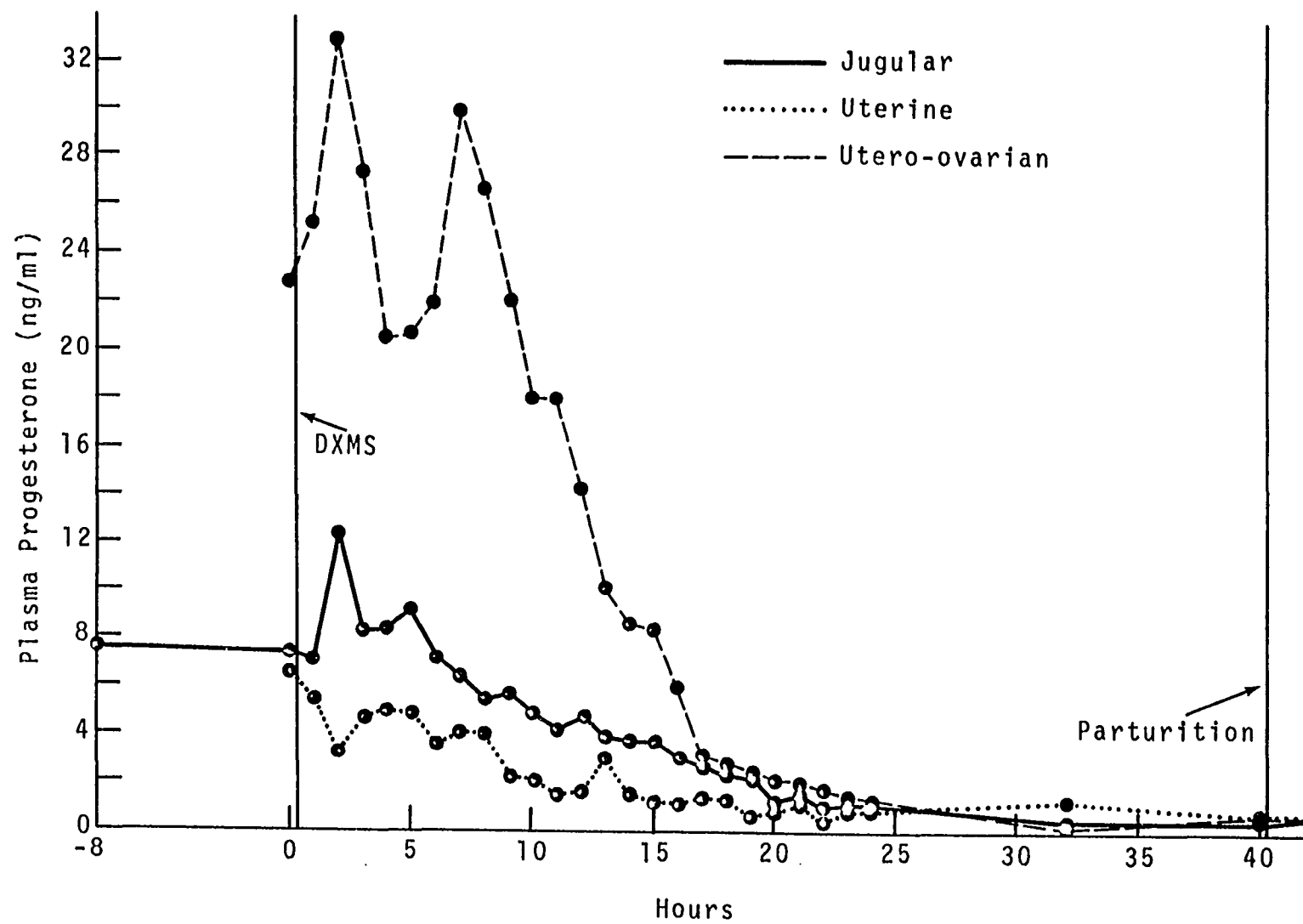


Figure 24. Plasma progesterone (ng/ml) in jugular, utero-ovarian and uterine veins of DXMS treated cows (n = 4)

First utero-ovarian and uterine samples were collected (0) just prior to DXMS treatment.





the uterine and jugular plasma and more rapidly in the utero-ovarian plasma until all samples were equal at 25 hours after DXMS injection. All samples averaged between 0.5 and 1 ng/ml for 12 hours prior to the average calving time of 42 hours after DXMS treatment.

The samples were tested statistically for significant differences in progesterone content between sample sites or between time of collection (Table 4). Using least squares analysis of variance, a significant difference ( $P < .01$ ) was found between sampling time. Equally significant ( $P < .01$ ) was the difference in progesterone content between the utero-ovarian and the jugular or uterine plasma. Interaction between sample site and sample time was found not to be significant.

Table 4. Plasma progesterone (ng/ml, mean  $\pm$  SE) in DXMS treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
	12.16 $\pm$ 2.35	7.86 $\pm$ 1.80	0.71 $\pm$ 0.10 <sup>b</sup>
Site <sup>c</sup>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>
	4.19 $\pm$ 0.82	3.47 $\pm$ 0.79	13.07 $\pm$ 2.80

<sup>a</sup>T<sub>1</sub> = 24 hours prior to DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24-48 hours after DXMS.

<sup>b</sup>Significantly different from T<sub>1</sub> and T<sub>2</sub> ( $P < .01$ ).

<sup>c</sup>S<sub>1</sub> = jugular v., S<sub>2</sub> = uterine v., S<sub>3</sub> = utero-ovarian v.

### 3. Estrogens

Jugular plasma estrogen levels in DXMS treated cows are shown in Figures 25 and 26. They are eight hour sample means of five cows. Plasma estrone averaged 500 pg/ml and estradiol averaged about 35 pg/ml prior to DXMS. Post-treatment levels of estrone and estradiol were about 560 and 50 pg/ml respectively through calving and fell rapidly the first 16 hours postpartum. Plasma estrone averaged 140 pg/ml and estradiol 14 pg/ml at 16 hours postpartum. Considerable variation occurred between individual cows as evidenced by the large standard errors in Figures 25 and 26, but all five animals showed an increase in plasma estrogens as parturition neared.

Individual eight hour samples of jugular plasma estrone in the control cow, which calved without DXMS, and cow 38, which failed to induce, are shown in Figure 26. Pre-calving estrone averaged about 1000 pg/ml for five days and rose rapidly the last 24 hours to a high of 1550 pg/ml just before the control cow calved. In contrast cow 38 had estrone levels considerably less than half the induced cows' levels and about one-fifth the levels of the control cow.

Jugular and utero-ovarian vein plasma from four cows were assayed for estrone content. Utero-ovarian levels averaged nearly threefold the jugular levels following DXMS treatment until calving (Figure 27). Jugular samples averaged 630 pg/ml after surgery and increased to 730 pg/ml at calving, about 48

Figure 25. Jugular plasma estradiol (pg/ml, mean  $\pm$  SE) in DXMS treated cows (n = 5)

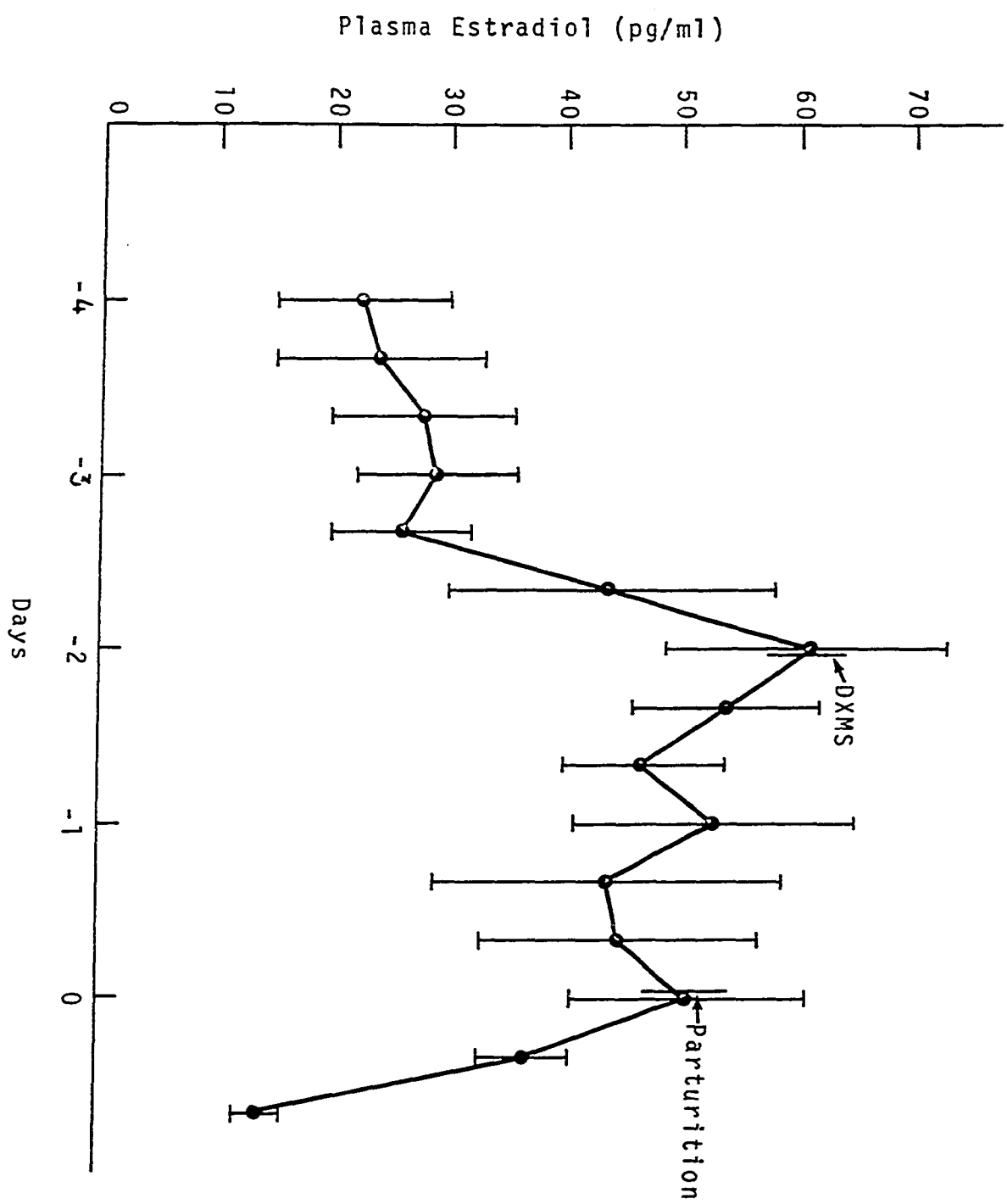


Figure 26. Jugular plasma estrone (pg/ml) in DXMS treated cows (C) calving, (NC) not calving and in naturally calving cow

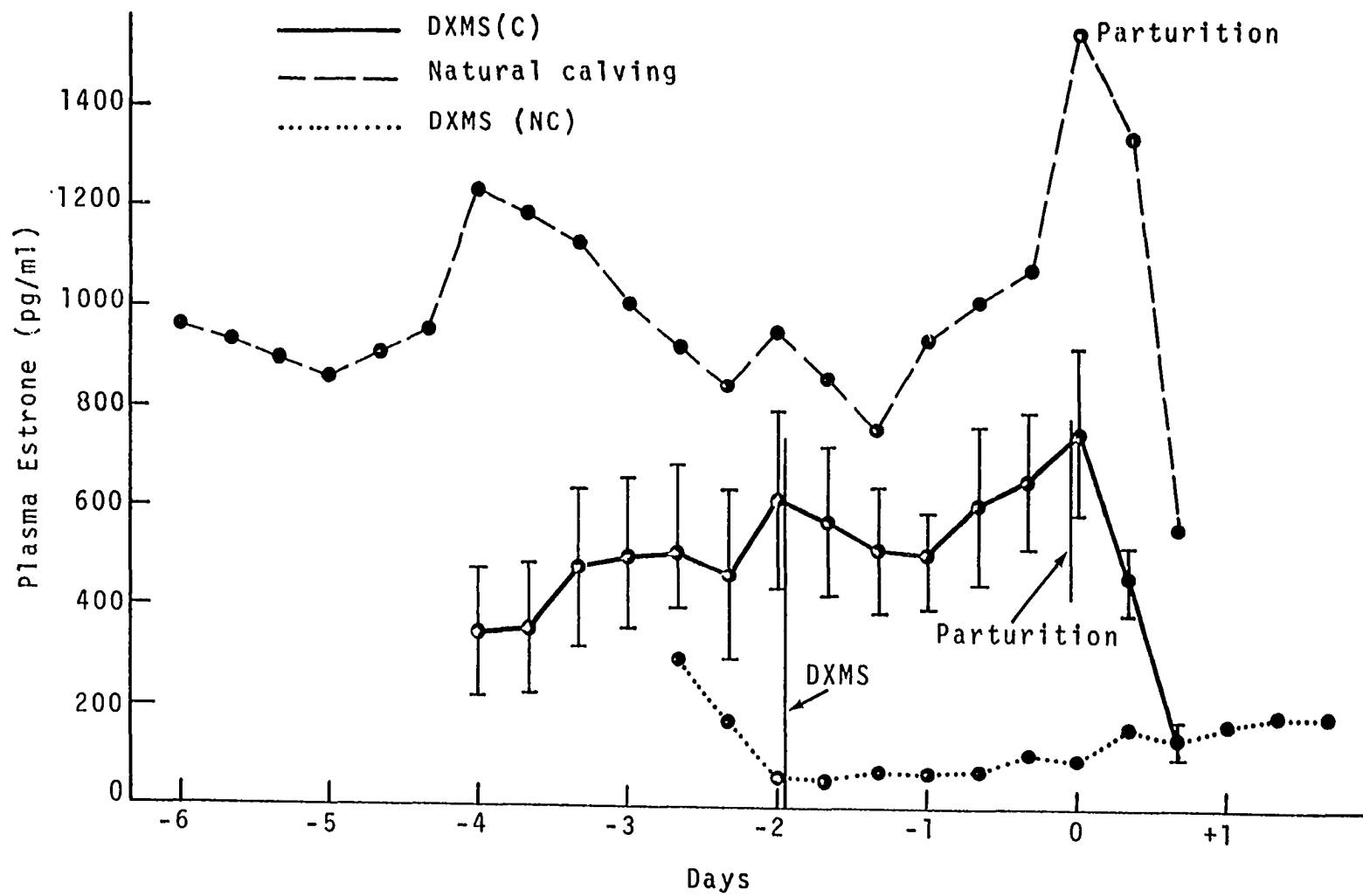
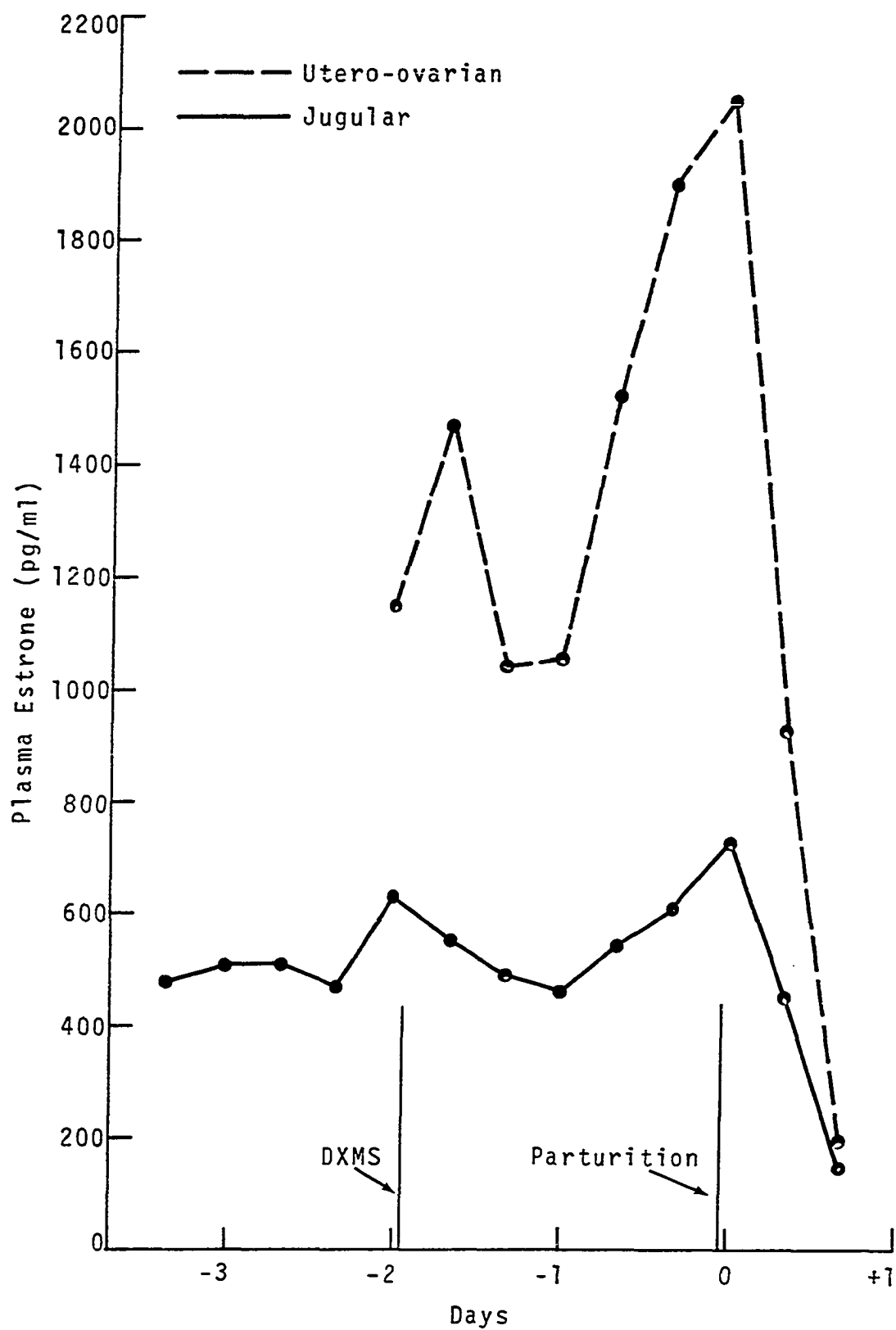


Figure 27. Plasma estrone (pg/ml) in jugular and utero-ovarian veins of DXMS treated cows before and after parturition (n = 4)





hours later. Utero-ovarian plasma estrone averaged 1150 pg/ml after surgery and rose to a peak of 2050 pg/ml 48 hours after DXMS treatment.

These sample averages were tested statistically, utilizing least squares analysis of variance, for significant differences in estrone content between the two sample sites and between the time of collection (Table 5). The 25 hours sample averages were not significantly different ( $P > .05$ ). There was a significant difference ( $P < .01$ ) between the means of jugular and utero-ovarian vein. Interaction between sample site and time was not significant.

Table 5. Plasma estrone (ng/ml, mean  $\pm$  SE) in DXMS treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
	843.2 $\pm$ 180.9	842.6 $\pm$ 175.3	1001.9 $\pm$ 262.7
Site <sup>b</sup>	S <sub>1</sub>	S <sub>3</sub>	
	553.0 $\pm$ 81.9	1538.8 $\pm$ 228.5 <sup>c</sup>	

<sup>a</sup>T<sub>1</sub> = 24 hours before DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24 hours after DXMS.

<sup>b</sup>S<sub>1</sub> = jugular v., S<sub>3</sub> = utero-ovarian v.

<sup>c</sup>S<sub>3</sub> = significantly different from S<sub>1</sub> ( $P < .01$ ).

#### 4. E:P ratio

The relationship of estrogens to progesterone prior to and following DXMS induced parturition in five cows is shown in Figure 28. Total plasma estrogens increased moderately and plasma progesterone decreased markedly after treatment. The estrogen:progesterone ratio (Figure 29) averaged nearly 0.1 prior to treatment and for 16 hours after DXMS. During the remaining 32 hours before calving the ratio increased rapidly to 2.1 at calving. An abrupt drop to below the pretreatment ratio occurred after calving.

The control cow which calved naturally, showed a similar pattern in estrogen:progesterone ratio near calving (Figure 29). A ratio of 0.2 occurred for days three and two prior to calving. There was a sharp increase in the last 32 hours to well above 2.0 just prior to calving.

#### C. Effects of HCG in DXMS Treated Cows

Six cows were treated with HCG<sup>1</sup>, (1,000 units every eight hours) beginning one day prior to and continuing 48 hours after DXMS treatment. Four cows, numbers 3, 4, 9 and 13, calved an average 52 hours after DXMS injection. Cows 5 and 11 did not calve. Cow 5 calved 11 days after DXMS and cow 11 delivered a stillborn calf with assistance six days post-DXMS.

---

<sup>1</sup>Follutein, E. R. Squibb & Co., New York, N.Y.

Figure 28. Jugular plasma estrogens (estrone and estradiol, pg/ml) and plasma progesterone (ng/ml) in DXMS treated cows before and after parturition (n = 5)

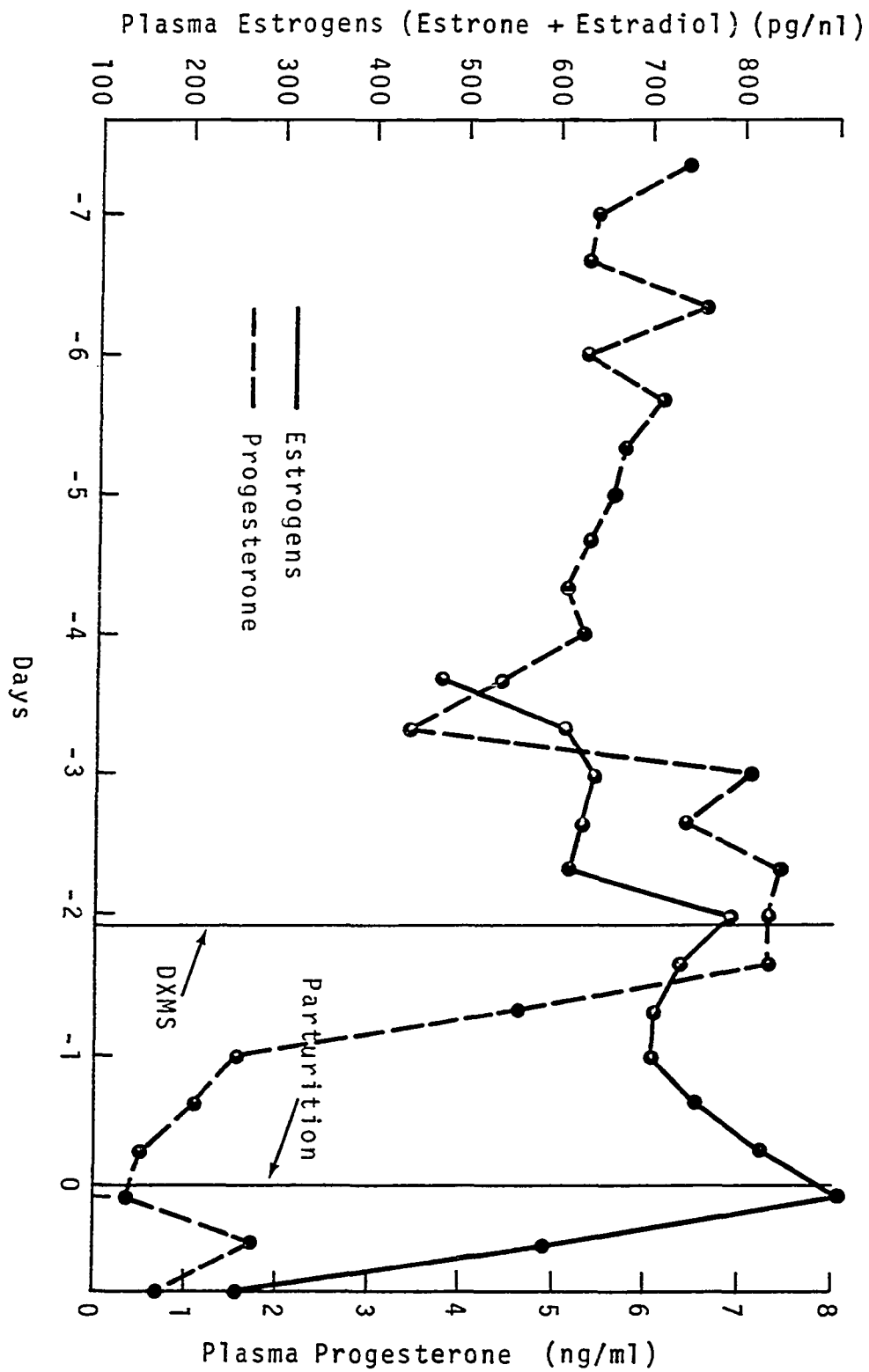
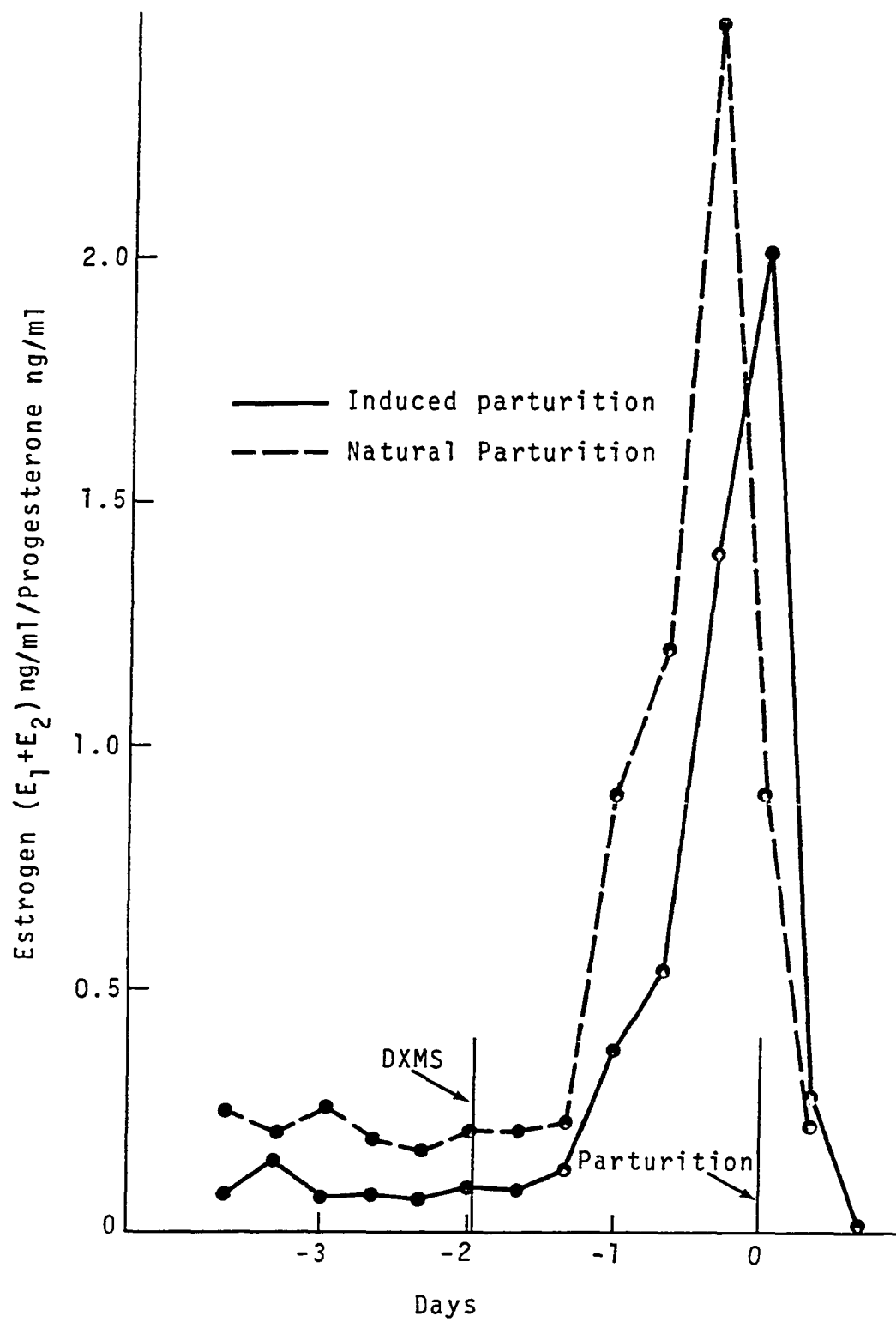


Figure 29. Jugular plasma estrogens:progesterone ratio in DXMS treated cows at parturition and in a naturally calving cow



Jugular plasma progesterone levels in the HCG treated cows are presented in Figure 30. The progesterone levels of five DXMS-induced cows are also included in Figure 30. HCG treated cows that were successfully induced averaged 6.8 ng/ml for 24 hours prior to HCG and 6.6 ng/ml for 24 hours after HCG treatment commenced. After DXMS, plasma progesterone levels declined slightly the first 24 hours to near 4 ng/ml and continued to decline to a mean of 0.8 ng/ml near calving. Cows 5 and 11 which failed to calve, averaged nearly 8 ng/ml for 96 hours, except for a brief drop to 3.6 ng/ml, 48 hours after DXMS.

Jugular plasma estrone levels for the same cows are shown in Figure 31. Cows 3, 4, 9 and 13, which calved after DXMS treatment regardless of HCG supplementation, averaged 865 pg/ml for 24 hours after HCG was started. A gradual rise in plasma estrone followed DXMS administration until the level peaked at 1410 pg/ml at calving.

Cows which failed to induce after HCG and DXMS injections had lower plasma estrone levels, averaging near 500 pg/ml, than the other four cows. Near the expected time of induced labor estrone levels had increased in these two cows to about 950 pg/ml and continued to rise to a peak of 1200 pg/ml, 72 hours after DXMS.

The mean plasma progesterone of all HCG-treated cows (Table 6) was moderately reduced during the 24-48 hour period

Figure 30. Jugular plasma progesterone in HCG and DXMS treated cows

Jugular plasma progesterone (ng/ml) in DXMS and HCG treated cows that calved (C). DXMS and HCG treated cows that did not calve (NC) and DXMS treated cows that calved. HCG, 3000 units per day in three divided doses, was injected subcutaneously for 3 days, starting 24 hours before DXMS treatment.



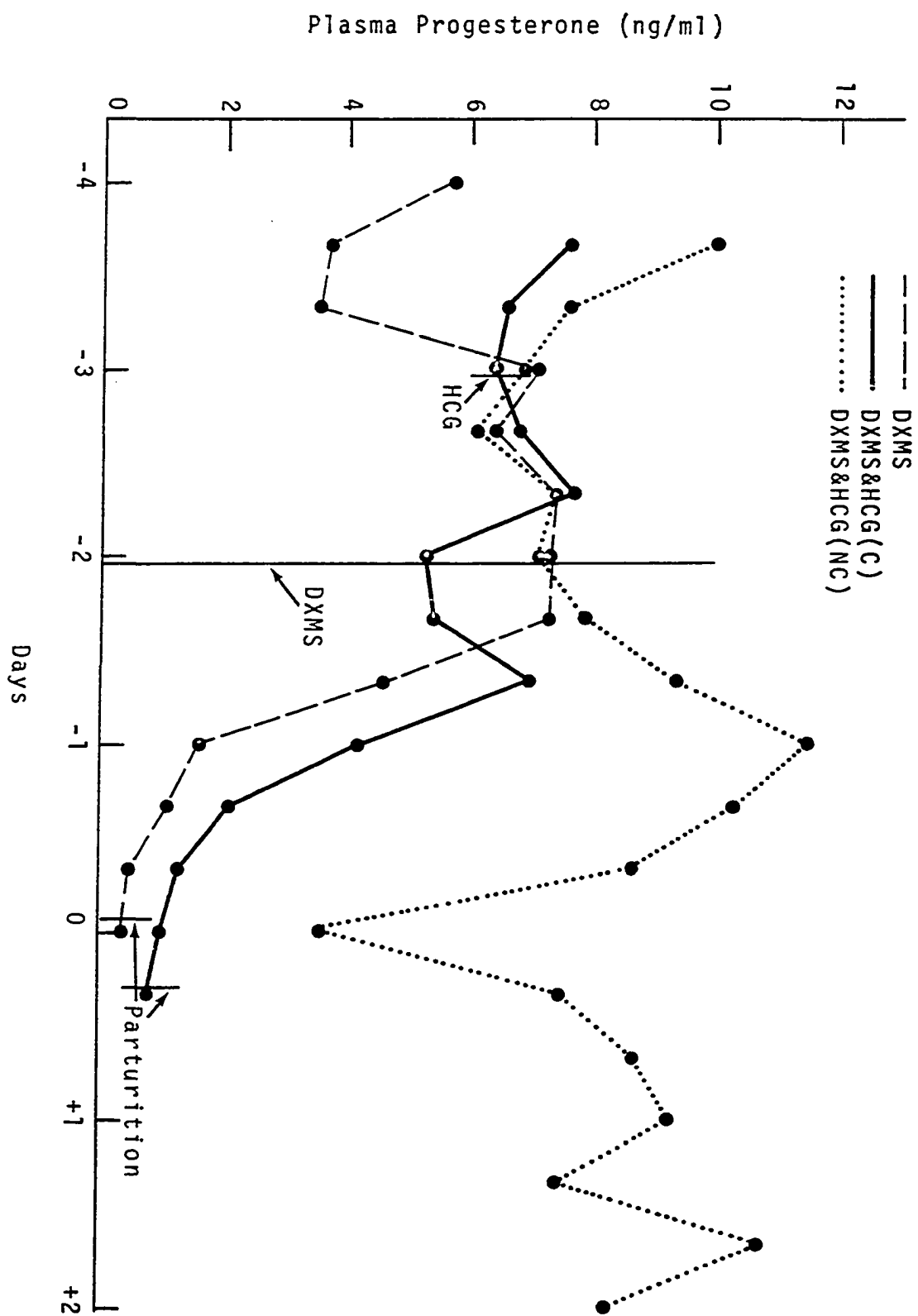


Figure 31. Jugular plasma estrone in HCG and DXMS treated cows

Jugular plasma estrone (pg/ml) in DXMS and HCG treated cows that calved (C), DXMS and HCG treated cows that did not calve (NC) and DXMS treated cows that calved. HCG, 3000 units per day in three divided doses, was injected subcutaneously for 3 days, starting 24 hours before DXMS treatment

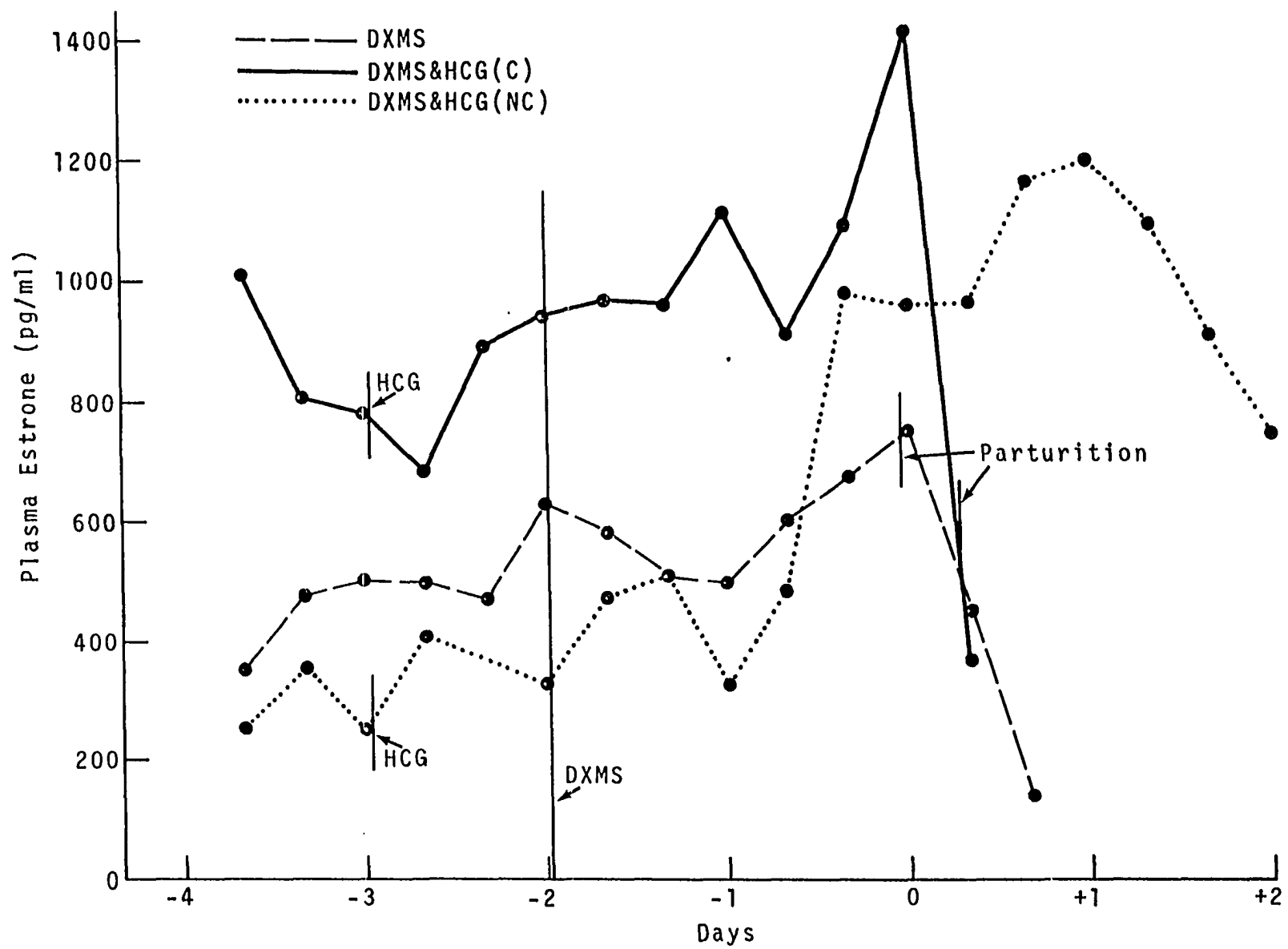


Table 6. Plasma progesterone (ng/m, mean  $\pm$  SE) in HCG treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
	6.62 $\pm$ 0.93	6.86 $\pm$ 1.15	3.73 $\pm$ 0.97 <sup>b</sup>
Calving <sup>c</sup>	C <sub>1</sub>	C <sub>2</sub>	
	4.28 $\pm$ 0.97	8.37 $\pm$ 1.04 <sup>d</sup>	

<sup>a</sup>T<sub>1</sub> = 24 hours before DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24 hours after DXMS.

<sup>b</sup>T<sub>3</sub> not significantly different (P > .05) than T<sub>2</sub> or T<sub>1</sub>.

<sup>c</sup>C<sub>1</sub> = cows that calved, C<sub>2</sub> = cows that failed to calve.

<sup>d</sup>C<sub>2</sub> is significantly different (P < .01) than C<sub>1</sub>.

after DXMS, but this reduction was not significant (P > .05). However, progesterone levels in cows calving within 72 hours of DXMS administration were significantly lower (P < .01) than cows that failed to respond.

Plasma estrone was not significantly altered over time by DXMS in HCG treated cows when data from all cows were pooled. However, estrone levels were significantly higher (P < .05) in cows calving following DXMS compared to treated cows which failed to respond to DXMS (Table 7).

Jugular plasma progesterone in the calving cows did decrease moderately after DXMS treatment (Table 8). An analysis

Table 7. Plasma estrone (pg/ml, mean  $\pm$  SE) in HCG treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
	718.3 $\pm$ 98.0	856.5 $\pm$ 115.9	882.3 $\pm$ 136.4
Calving <sup>b</sup>	C <sub>1</sub>	C <sub>2</sub>	
	925.7 $\pm$ 96.7 <sup>c</sup>	605.8 $\pm$ 82.2	

<sup>a</sup>T<sub>1</sub> = 24 hours before DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24-48 hours after DXMS.

<sup>b</sup>C<sub>1</sub> = cows that induced, C<sub>2</sub> = cows that failed to induce.

<sup>c</sup>C<sub>1</sub> significantly different (P < .05) from C<sub>2</sub>.

Table 8. Plasma progesterone (ng/ml, mean  $\pm$  SE) in HCG treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Calved (4)	8.32 $\pm$ 0.3	5.5 $\pm$ 1.14	1.25 $\pm$ 0.27 <sup>b</sup>
Not calved (2)	6.83 $\pm$ 1.13 <sup>c</sup>	9.58 $\pm$ 1.51	8.63 $\pm$ 1.40

<sup>a</sup>T<sub>1</sub> = 24 hours after HCG, before DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24 hours after DXMS.

<sup>b</sup>T<sub>3</sub> not significantly different (P > .05) from T<sub>1</sub> and T<sub>2</sub>.

<sup>c</sup>T<sub>1</sub> not significantly different (P > .05) from T<sub>2</sub> and T<sub>3</sub>.

of variance showed this decrease approached, but was not significant ( $P > .05$ ). Cows 5 and 11 which failed to induce after DXMS treatment showed a moderate but non-significant ( $P > .05$ ) increase in plasma progesterone after DXMS injection (Table 8).

Both of the above mentioned groups were analyzed statistically for significant changes in plasma estrone during the 24 hours preceding the 48 hours following DXMS (Table 9). Neither group had significant ( $P > .05$ ) changes in plasma estrone at the various testing intervals.

Table 9. Plasma estrone (pg/ml, mean  $\pm$  SE) in HCG treated cows

Time <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Calved (4)	718.3 $\pm$ 98.0	1014.7 $\pm$ 141.8	882.3 $\pm$ 75.5
Not calved (2)	467.5 $\pm$ 79.2	540.0 $\pm$ 87.5	810.0 $\pm$ 128.1 <sup>b</sup>

<sup>a</sup>T<sub>1</sub> = 24 hours after HCG, before DXMS, T<sub>2</sub> = 24 hours after DXMS, T<sub>3</sub> = 24-48 hours after DXMS.

<sup>b</sup>T<sub>3</sub> not significantly different ( $P > .05$ ) from T<sub>1</sub> and T<sub>2</sub>.

## V. DISCUSSION

## A. Postpartum Reproductive Function

Gross measurements either at slaughter or via rectal palpation indicated that induced parturition delayed uterine involution in the early postpartum period, but these cows appeared to catch up in the latter postpartum period.

Uterine horn diameters of two cows slaughtered at 14 days were slightly larger (9.8 and 5.6 cm) than those estimated by rectal palpation in the 16 cows which retained fetal membranes (8.3 and 4.7 cm). Uterine horn size in those cows which did not retain the fetal membranes averaged 6.8 and 3.8 cm in diameter for the prior gravid and non-gravid horns. These results are similar to those reported by Morrow et al. (1966) for normal cows and cows with retained placentae.

There was no appreciable difference in uterine sizes between cows with or without retention of the fetal membranes by 30 day postpartum. The prior gravid and non-gravid horns measured 3.8 and 2.5 cm in diameter respectively. These results agree quite well with Morrow et al. (1966) and Wagner and Hansel (1969) although the latter reported that nursed cows were approximately 1 cm larger across each horn than milked cows at 30 days postpartum. Uterine horn sizes at 42 days postpartum were 3.1 and 2.7 cm respectively. These measurements were comparable to those reported by Morrow et al. (1966) for 60 day

postpartum cows.

A review of the literature by Morrow, Roberts and McEntee (1969) indicated that physical involution of the uterus is complete by 42 days. Buch, Tyler and Casida (1955) and Morrow et al. (1966) suggested that cows with abnormal parturition or placental retention are delayed at least five days. Rasbech (1950) suggested the process may only require 20 to 25 days in the normal cow. The similarity in uterine size between cows which had placental retention and those which did not suggest that normal involution is accomplished sometime before 30 days postpartum. Cattle with placental retention may achieve a similar degree of involution by 35 days postpartum.

Uterine weight also decreased rapidly from two weeks to four weeks postpartum. A slight increase in uterine weight occurred after four weeks. This increase coincides with increased thickening of the endometrium.

Rasbech (1950) and Morrow et al. (1966) found that uterine lochia was nearly absent in normal cows by 14 days postpartum. However, cows with retained placentae had as much as 300 ml at that time. Slaughtered cows in this study averaged 460 ml at two weeks and 30 ml at four weeks postpartum. The fact that the fetal membranes were retained until 8 or 10 days accounts for the increased uterine lochia.

Ovarian activity was assessed primarily at slaughter or by rectal palpation. Only one of 29 cows was observed in



estrus prior to 28 days and 3 of 26 cows were observed in estrus by six weeks postpartum. Cyclic corpora lutea were detected in two of three slaughtered cows and 7 of 23 palpated cows by six weeks after calving. Milked cows generally have first ovulation and often a detectable estrus by 13 to 25 days postpartum (Morrow et al., 1966 and Wagner and Hansel, 1969). Wiltbank and Cook (1958) noted that nursed beef cows averaged 53 days until first ovulation and 85 days until first estrus after calving. More recently Wagner and Oxenreider (1971) observed that suckled cows averaged 52 days to first postpartum ovulation and 56 days until first estrus. The ovarian function detected in this study by 42 days postpartum appears to be well within the limits described by other studies as normal for nursing cows.

The histological process of uterine involution followed the descriptions of Rasbech (1950), Gier, Singh and Marion (1962) and Gier and Marion (1968). Cows which calved after DXMS treatment proceeded in a similar fashion, although retention of the fetal membranes altered the rate of involution (Figure 19).

Epithelization of the caruncular surface began at the intercaruncular granular epithelium and spread up and over the denuded caruncle. In normal nursed cows and DXMS treated cows which calved without placental retention the caruncles were normally covered by epithelium at 28 days postpartum. At this stage the epithelium over the upper portions of the

caruncles appeared as very flat cells. By 42 days, the surface cells were normal columnar epithelium. Epithelization of the caruncle was delayed when the fetal placenta membranes were retained. This delay was most noticeable at 30 days postpartum, because many of the caruncles in these cows were not fully covered with epithelium. However histological sections from 42 day postpartum cows, whether they had placental retention or not, had normal columnar epithelium over the caruncles. The delay in re-epithelization of the caruncle coincided with the occurrence of uterine inflammation.

In this study retained fetal membranes were associated with uterine inflammation. Such cows had considerable exudate in the lumen of the uterus at 14 days postpartum and necrosis of the intercaruncular epithelium was common. Exudate was observed to cover the caruncular surface and excessive numbers of polymorphonuclear cells were observed in the caruncular stroma.

Wagner and Hansel (1969) observed that exudate or infection delayed re-epithelization of the caruncle. The new epithelium appeared to attach poorly to the underlying stroma and frequently was curled back.

Caruncular resolution involves the removal of the maternal placenta and a reduction in caruncular size. Early this process involves the sloughing of the placental mass down to the caruncular crypts and is usually complete by 10 days postpartum. The rapid contraction of the caruncular blood vessels

and reduced blood flow causes necrosis of the caruncular mass and shortening of the caruncular stalk. Further resolution of the caruncle is more gradual and involves the removal of nonfunctional vascular and supportive tissues. Large reactive histiocytes are numerous in the stratum compactum and stratum spongiosum from 15 to 40 days postpartum. These cells are usually gone by 60 days (Gier and Marion, 1968). Neutrophils accumulate in the sloughing placental mass and to some extent in the upper stratum compactum during the first 2 weeks, but are noticeably absent at four weeks in the caruncles of normal cows. DXMS treated cows which had placental retention had more neutrophils in the caruncular mass than the normal cows at two weeks postpartum. Cryptal giant cells were evident in cows which had not sloughed the placental decidua. Wagner and Hansel (1969) identified these giant cells located just outside the line of demarcation in their study of uterine involution. Björkman (1970) referred to the multinucleated cryptal giant cells of the uterine epithelium. He differentiates these from the trophoblastic giant cells in the cow which have one or two nuclei. He did not define a function for cryptal giant cells.

Lymphocytic foci were not apparent in the involuting caruncle at two weeks postpartum, but they were frequently seen at four weeks postpartum. They were less evident at six weeks after calving. Uteri which appeared to have more than

normal numbers of neutrophils at six weeks postpartum, also had more lymphocytic foci. Caruncular resolution in all the DXMS treated cows was usually equal to that seen in normal cows.

Examination of the intercaruncular surface at postpartum was usually not a reliable index of uterine involution in the normal cow. The 14 day postpartum uterus was distinguishable from the four and six weeks histological sections by the presence of neutrophils, vacuoles in the epithelial cells and focal areas of epithelial hyperplasia but the intercaruncular epithelium in four and six week postpartum uteri were similar in appearance. This is in agreement with Gier and Marion (1968) and Wagner and Hansel (1969).

Uterine inflammation following placental retention caused an increased accumulation of neutrophils in the epithelium at 14 day postpartum. By four weeks the only evidence of a previously retained placenta was a slight increase in neutrophils and increased numbers of lymphoid follicles within the lamina propia.

The degree of neutrophil infiltration of the endometrium closely parallels the rate of endometrial regeneration. In the normal cow polymorphonuclear cells generally decrease in the endometrium after 10 days postpartum (Rasbech, 1950 and Riesen et al., 1968). Gier and Marion (1968) reported neutrophils were numerous in infected cows at 30 days postpartum. In

this study DXMS treated cows had more neutrophils accumulated in the endometrium and on its surface at 14 days postpartum than normal cows. Cows which had had prior retained placentae continued to have more than normal numbers of neutrophils in the endometrium when examined at four and six weeks after calving.

Regeneration of the endometrial glands did not appear to be influenced by the rate of uterine involution. They were scant in number at two weeks, but by four weeks they were more numerous. At six weeks the endometrium was filled with coiled and branching glands. Those uteri with acute inflammation at two weeks postpartum often had endometrial glands obstructed with tissue debris.

Wagner and Hansel (1969) found that ovarian activity apparently increased the rate of endometrial gland development after calving. They concluded, however, that normal ovarian activity was not essential to the overall process of involution.

Oxenreider and Wagner (1971) observed that lactation, milking or suckling, and low nutrition significantly reduced the uterine weight of 56 day postpartum cows. A portion of this reduction was related to the incidence of ovulation in lactating cows. Cycling lactating cows had significantly larger and heavier uterine horns compared to non-cycling lactating cows, but non-lactating cows had uterine horns that

were larger and heavier than lactating cows. Riesen et al. (1968) attributed the smaller uterine horns to nursing and suggested on the basis of myometrial cell size and number that the myometrium rather than the endometrium was the principal area affected. Oxenreider and Wagner (1971) also demonstrated a significant decrease in myometrial cell size in the animals that had not experienced postpartum cyclic ovarian activity.

Myometrial involution was influenced in the early postpartum period by acute metritis. In such cases the myometrial cells were enlarged, and often contained a hyaline staining granular cytoplasm. Examinations at subsequent intervals revealed myometrial involution was largely independent of the endometrial stages of involution. Myometrial involution was basically a two phase process of muscle contraction and atrophy. There was little evidence of myometrial autolysis although a few cells appeared to have small darker staining nuclei and more eosinophilic staining cytoplasm at four weeks postpartum. At six weeks the myometrial cells were smaller and tighter packed, but appeared more uniform in size and staining characteristics.

Resolution or involution of the vascular system in the postpartum uterus precedes many of the other tissue changes and appears to be largely independent of their progress.

Rasbech (1950) concluded that vasoconstriction resulted in shortening of the caruncular stalk and dissolution of the

decidua. Both processes are usually complete by 9 or 10 days postpartum. Gier and Marion (1968) observed that the caruncular vessels were nearly occluded by two days postpartum.

In the seven day uteri thrombosed vessels were frequently seen extending into the caruncular mass. By 14 days the vessels in the myometrium and caruncular base were contracted and tortuous. The intima was thrown into folds which protruded into the vessel lumen and there appeared to be an increase in connective tissue immediately under the endothelium. The media of some vessels appeared to be undergoing hyaline degeneration and connective tissue replacement. This process occurred primarily in the caruncular vessels. The deeper larger vessels that coursed through the myometrium underwent a process of contraction which formed thick walled and tortuous vessels. The smooth muscle cells of these arteries appeared to atrophy, but they did not show the degenerative changes seen in the septal arteries. The intimal layer of these larger vessels also appeared thickened with connective tissue.

Myometrial, vascular and glandular changes appeared to proceed normally in the DXMS treated cows regardless of the degree of inflammation in the endometrium. Conversely, endometrial resolution was very much affected in the early postpartum period by the degree of inflammation, which was more acute in this study when the placenta was retained. Caruncular

resolution and re-epithelization proceeded much like the process of wound healing. Exudate appeared to retard re-epithelization of the caruncle.

In the normal cow, endometrial resolution is complete between 25 and 30 days (Rasbech, 1950; Gier and Marion, 1968; Wagner and Hansel, 1969). In this study cows with placental retention often lacked complete epithelization of the caruncle and had more neutrophils in the endometrium than normal cows. By 42 days it was impossible to differentiate between DXMS treated cows and normal cows on the basis of histological sections.

If the major criteria for complete uterine involution are good glandular development and an intact mucosal epithelium, then the DXMS treated animals were nearly involuted by four weeks and had completed involution before six weeks postpartum. Assuming an average interval of 42 days from calving to completed involution, DXMS treated cows achieve normal uterine involution about the time that normal cyclic estrus returns in milked cows and in advance of the commencement of cyclic estrus in nursed cows (Wiltbank and Cook, 1958; Menge et al., 1962; Morrow et al., 1966; Wagner and Oxenreider, 1971).

Postpartum fertility and uterine involution in other parturition induction studies are consistent with the results in this study.



Adams and Wagner (1970) reported that 27 of 46 cows induced with DXMS had retained fetal membranes. Endometrial biopsy at 14-19 days postpartum indicated at least a mild degree of inflammation. However, epithelial regeneration appeared to be normal.

Lauderdale (1972a) reported that induced cows had a conception rate of 70% versus 79% in controls. The mean interval between calving and conception was  $59 \pm 5$  (mean  $\pm$  S.E.) days in treated cows and  $55 \pm 3.3$  days in controls. However, control cows had a mean interval of only 23 days between calving and the start of the breeding season and induced cows had a mean interval of 42 days.

Wagner, Willham and Evans (1971) found that pregnancy occurred in 104 of 154 cows (67.5%) with retained placentae versus 106 of 142 cows (74.6%) that did not retain the placentae. Mean services per conception were 1.23 and 1.35, respectively, for cows retaining the placentae and cows without placental retention. Although no severe cases of metritis were evident, cows with a retained placenta were given 4 million U. penicillin and 5 g streptomycin intramuscularly the day following parturition.

It appears that given proper postpartum care and sufficient time for postpartum involution, the cow has normal reproductive functions following DXMS induced parturition. In most of the above studies the induced cow had an additional

one to two weeks compared to the normal cow in calving to breeding interval. This one to two weeks represents the additional time afforded by the induction procedure.

#### B. Relationship of DXMS Treatment and Steroid Changes at Parturition

A second objective of this study was to elucidate the changes in circulating steroid levels following DXMS treatment in late term cows and to relate these changes to the occurrence of parturition. Why do some cows induce successfully with DXMS and other do not? Alterations in blood steroid levels are an important aspect to be considered in this question.

Exogeneous glucocorticoids at dosages sufficient to elicit parturition in the cow caused an absolute drop in endogenous plasma corticoids to less than 1 ng/ml for 48 to 72 hours after treatment (Adams and Wagner, 1970). In this study the drop in plasma corticoids levels following DXMS was not as great nor as long, but these cows had been subjected to abdominal surgery for placement of internal catheters. Immediately following surgery, plasma corticoids averaged nearly 15 ng/ml, an increase of about 7 ng/ml from pre-surgery levels. DXMS treatment was initiated immediately after the first blood collection following surgery so that the cows' corticoid response to surgery would augment the exogenous corticosteroids.

Within 16 hours after DXMS treatment, corticoid levels in all cows had decreased to about 3.5 ng/ml. Thereafter a

general rise in plasma corticoids occurred until parturition. There was, however, considerable variation in this response among individual cows. One cow responded with high plasma corticoids while two other cows showed no increase as parturition neared.

Plasma corticoid levels in samples collected from the uterine or utero-ovarian vein were less than jugular levels. This would suggest that fetal corticoids were not passing the placenta and contributing to maternal corticoid levels or that the exogenous corticoids may have suppressed any such contribution by the fetus.

In the fetal lamb there is a several-fold increase in the plasma concentration of cortisol during the last three to four days before delivery (Bassett and Thorburn, 1969; Thompson, 1973), but in the ewe, maternal corticoids do not show a significant increase until shortly before parturition (Thompson, 1973). Fetal hormone studies are lacking in the bovine, but Balfour (1953) and Eberhart and Patt (1971) demonstrated that cortisol levels were high in the neonate calf and much lower by two to three days after birth.

In the pregnant cow plasma corticoids increased slightly the last five days and nearly doubled on the day of parturition (Adams and Wagner, 1970; Smith et al., 1973). The control cow in this experiment followed a similar blood corticoid pattern.

A notable feature encountered in all the cows that calved within 72 hours after DXMS treatment was a precipitous drop in plasma progesterone for at least 24 hours before calving. Pre-treatment jugular plasma progesterone levels averaged near 6 ng/ml. Within 24 hours after DXMS treatment jugular plasma progesterone averaged near 1 ng/ml and remained at this level beyond calving. Cows which failed to calve had no appreciable decline in plasma progesterone after treatment. Jugular plasma progesterone in the control cow averaged 4 ng/ml from six to two days prior to calving and about 1 ng/ml the remaining 48 hours before calving. The results in this study agree quite well with progesterone studies in the normal cow (Donaldson, Bassett and Thorburn, 1970; Smith et al., 1973; Edqvist et al., 1973) and in DXMS treated cows (Wright et al., 1970; Edqvist et al., 1972).

In other studies, DXMS treated cows which did not calve (Wright et al., 1970) and cows with prolonged gestation (Holm and Short, 1962) did not have a significant drop in peripheral plasma progesterone at the proper time for calving. It is apparent that an abrupt drop in peripheral plasma progesterone is an important component of normal parturition in the cow.

Less settled is the question as to the source of maternal plasma progesterone during pregnancy in the cow. Based on ablation studies, McDonald, McNutt and Nichols (1953) suggested that the corpus luteum of pregnancy was necessary until

approximately the 200th day. Thereafter, individual variation occurred in the dependency for the corpus luteum.

More recent studies have shown the corpus luteum of pregnancy to be functional throughout pregnancy (Stormshak and Erb, 1961; Edwards, 1962; Estergreen et al., 1967; Erb et al., 1968). It is during the last month of gestation that the cow is particularly dependent upon an ovarian source of progesterone.

Progesterone concentration in the utero-ovarian vein was 2.5 to 3 times higher than in the jugular and uterine veins of the four cows subjected to internal catheterization (Figure 24). This difference was apparent both after surgery and DXMS treatment. Uterine vein samples were consistently lower than samples from the jugular and utero-ovarian veins. The ovary, presumably the corpus luteum, was therefore a primary contributor to peripheral plasma progesterone. The gravid horn of the uterus removed small quantities of progesterone from the peripheral circulation. One might conclude from these data that the placenta does not secrete sufficient progesterone to influence peripheral progesterone levels although it may secrete progesterone which is utilized within the uterus itself.

Plasma estrogens were found to increase moderately after DXMS treatment. Jugular estrone averaged 450 pg/ml during two days pre-treatment and rose to 750 pg/ml at parturition

40-48 hours after DXMS. In the normal cow, plasma estrone was roughly two times as high as DXMS treated cows. After calving estrone levels declined sharply. Cows which failed to include with DXMS had significantly lower plasma estrone levels, though these levels increased slightly after DXMS administration.

Plasma estradiol levels were about one-tenth the estrone levels and also decreased sharply after parturition. Estrone and estradiol levels in this study approximate the levels reported by Arije, Wiltbank and Hopwood (1971); Henricks et al. (1972); Edqvist et al. (1972) and Edqvist et al. (1973). Plasma estrogens increase in a similar manner in the sow (Molokwu and Wagner, 1973); the ewe (Challis, Harrison and Heap, 1971a) and the goat (Thorburn et al., 1972) as parturition approaches.

Differences in estrone concentration in utero-ovarian and jugular veins (Figure 27) indicate a uterine site of estrogen biosynthesis. Uterine vein samples assayed in two cows (not illustrated) were equal to utero-ovarian vein samples in estrone content. Placental synthesis of estrogen is known in primates (Diczfalusy, 1964) and in some other mammals (Davies, Ryan and Petro, 1970). Similar differences between uterine vein and peripheral plasma estrogen content are reported for the ewe (Bedford et al., 1972; Thompson, 1973) and the goat (Thorburn et al., 1972).

Since both estrogen and progesterone levels change as parturition nears, they are conveniently compared as a ratio. In this study the change in the estrogen:progesterone ratio dramatizes the profound change in circulating steroids at calving (Figure 29). The E:P ratio is greater in naturally calving animals (2.5 vs. 2.0) and the peak level occurs almost eight hours earlier. Based on steroid levels in previously cited reports the ratio is considerably higher in the natural calving cows as compared to DXMS treated cows in this study. The primary difference in the two reflect a higher estrogen levels in natural calving cows. Molokwu and Wagner (1973) and Thompson (1973) have determined similar ratios in the sow and ewe at parturition.

Successful induction of labor in cows with 20 mg DXMS was dependent upon at least two factors: First, plasma progesterone and its production or release from the corpus luteum must fall to minimum levels for at least 12 hours before calving. Second, plasma estrogen (estrone) levels must be near or above 400 pg/ml at the time of DXMS treatment.

Several investigators have tested the necessity of progesterone withdrawal for the commencement of labor. Exogenous progesterone did not delay the onset of labor in the ewe (Bengtsson and Schofield, 1963; Liggins et al., 1972) but it sometimes caused a protracted delivery. In the latter study uterine and plasma progesterone levels remained at near normal

levels even in the face of ACTH or glucocorticoid administration to the fetus.

Jöchle et al. (1972) administered 100 mg progesterone/day for 2 days preceding and three days after the injection of 10 mg flumethasone. Calving was prevented in these cows until after exogenous progesterone withdrawal. Dystocia and still-born calves were common at calving. DXMS induced parturition can be blocked in the sow with exogenous progesterone injections (First and Stagmiller, 1972).

Since the latter two species are dependent upon a luteal source of progesterone, it was theorized that DXMS might alter luteotropic support by the pituitary. A relationship between adrenocorticotropin (ACTH) or adrenal corticoids and pituitary gonadotropins is suggested in several studies. Liptrap and Raeside (1968) reported decreased output of luteinizing hormone in the boar after administration of ACTH or adrenal corticoids. ACTH administration during day 2 to 8 of the estrous cycle suppressed corpus luteum (CL) formation in the cow (Brunner, Donaldson and Hansel, 1969; Wagner, Strohbehn and Harris, 1972). Synthetic corticosteroids at the time of menstruation or during early follicular development blocked ovulation and shortened the interval to next menstruation in baboons (Hagino, 1972).

Experiment III was designed to test the theory that DXMS diminished progesterone production in the CL by altering



luteotropin support from the maternal or possibly the fetal pituitary. Human chorionic gonadotropin (HCG) given subcutaneously (1000 units/8 hours) for 24 hours preceding and 48 hours after DXMS treatment delayed parturition in two of six cows. All the cows prepared for parturition with relaxation of the vulva and pelvic ligaments and filling of the udder, but cervical dilation did not occur in the two cows which were delayed. One of these cows calved 11 days later and the other delivered a stillborn calf with assistance six days after DXMS administration.

Plasma progesterone levels were not significantly altered by HCG in the 24 hours prior to DXMS treatment (Figure 30). The cows which calved after HCG and DXMS calved about eight hours later than those receiving DXMS alone. Those cows which did not calve after HCG and DXMS treatment had significantly higher plasma progesterone than those which calved. Delayed parturition in the two cows paralleled the response described by Jöchle et al. (1972) following progesterone and DXMS treatment in late term cows.

Mills and Morrisette (1970) demonstrated that the bovine corpus luteum of pregnancy had increased progesterone secretion when perfused with LH during in vitro studies. Ovaries of late pregnancy responded equally as well as ovaries of early pregnancy and progesterone output nearly doubled after LH perfusion. This study did not demonstrate such a response

in vivo.

During pregnancy, plasma LH levels apparently change very little, remaining near 0.7 to 0.8 ng/ml (Randel and Erb, 1971; Oxender, Hafs and Edgerton, 1971). This would indicate a tonic regulation of luteal function during pregnancy. Schams et al. (1972) sampled pregnant cows every six hours and found LH levels were consistently low (1.0 to 1.6 ng/ml plasma) with only a few irregular peaks. More importantly, plasma LH levels were unchanged during normal or flumethasone induced parturition. This finding would seem to invalidate the theory of DXMS inhibition of luteotropin to induce the parturition. It does not exclude the possibility of a competitive interaction between luteotropin and a luteolytic agent which is directly or indirectly influenced by DXMS treatment.

Administration of HCG did not significantly alter plasma estrone levels (Figure 31). Estrone levels rose after DXMS even though plasma progesterone remained at high levels in the cows which did not calve.

The mechanism of DXMS induced parturition is still unsettled. Though quantitative differences exist it is significant that the major endocrine changes recognized in the cow before natural parturition also occur before premature parturition induced with exogenous glucocorticoids.

Experimental evidence is lacking in the bovine but evidence in the sheep and goat indicate the fetus to be a more

effective route of induction (Liggins, 1969a; Liggins et al., 1972; Thorburn et al., 1972). Synthetic glucocorticoids apparently mimic the fetal cortisol rise that occurs in natural parturition. Maternally administered corticosteroids cause premature labor if large doses are given late in pregnancy when placental permeability to corticoids is increased and estrogen synthesis by the feto-placental unit has developed to a significant degree. In the ewe subthreshold doses of DXMS (4 mg/day) caused no change in progesterone concentrations (Fylling, 1971). Liggins et al. (1972) infused DXMS (4 mg/day) into fetal lambs and parturition occurred in an average of 56 hours.

While the fetal pituitary-adrenal component of the mechanism initiating parturition in the cow is probably similar to that demonstrated for the sheep, the maternal progesterone changes may not be the same. The major site of progesterone production in the ewe in late pregnancy is the placenta (Linzell and Heap, 1968) while the cow is more dependent upon the corpus luteum of pregnancy. In both species maternal plasma progesterone falls and estrogen rises in the last few days preceding labor. How cortisol or exogenous glucocorticoids cause these steroid changes is still unclear.

Anderson et al. (1972a) demonstrated that pregnenolone or progesterone are readily transformed in the fetal adrenal to cortisol. Both steroids may also be transformed to C<sub>19</sub>

steroids which are significant precursors for placenta estrogen synthesis. The latter steroid pathways become significant in late pregnancy (after 130 days) in the ewe (Liggins et al., 1972). Whether DXMS blocks cortisol synthesis and diverts progesterone or pregnenolone to  $C_{19}$  steroid synthesis or alternately stimulates adrenal synthesis of the  $C_{19}$  steroids is unknown. However, DXMS given to late term cows or ewes results in an increase in maternal estrogens. DXMS before 130 days of pregnancy failed to cause increased levels of plasma estrogens in the ewe or fetus (Liggins et al., 1972). Edqvist et al. (1972) observed no increase in maternal plasma estrogens after DXMS treatment in cows from 75 to 150 days pregnant. When estrogen levels failed to rise, more DXMS was required to diminish plasma progesterone levels in the dam and elicit an abortion. Edqvist et al. (1972) observed that three of four cows in early pregnancy showed neither a rise in plasma estrogen or a drop in plasma progesterone in the dam after DXMS. However, DXMS at 40 mg/day for 7 to 12 days resulted in fetal death. It appears that a fetal age sufficient to support estrogen production or some yet unknown agent is necessary for DXMS induction of parturition. Nathanielsz et al. (1972) demonstrated that ACTH given to the 110 day fetal lamb over a period of several days enhanced adrenal production of cortisol and  $C_{19}$  steroids and caused maturation of the fetal adrenal.

Maternal estrogen levels appear to influence the length of gestation in cattle. Hunter et al. (1970) found that cows with longer gestations had lower levels of urinary estrogens. Similarly, cows with the high plasma estrogens had shorter gestation periods than cows with medium or low levels of plasma estrogen (Henricks et al., 1972).

Exogenous estrogens have been reported to cause premature parturition in sheep (Hindson, Schofield and Turner, 1967) and cattle (Gronborg-Pedersen, 1969). Adams (1969) could not cause parturition in late term cows with pharmacological doses of estrogen. Liggins et al. (1972) demonstrated that administration of DXMS or ACTH to the fetal lamb can cause premature parturition without a significant change in plasma estrogen. This does not support the suggestions of Challis (1971) that the peak of maternal plasma estrogens triggers parturition in the ewe.

The efficacy of DXMS in this study was related to maternal estrogen levels. Cows with plasma estrone levels below 400 pg/ml failed to calve after one injection of 20 mg DXMS.

Another dimension, not studied in this experiment, is the role of prostaglandins (PG) in natural or induced parturition.  $\text{PGE}_2$  or  $\text{PGF}_{2\alpha}$  were found to be an effective abortifacient in pregnant women as early as 1967 (Karim, 1968). The mechanism in women is believed to be a direct action on the myometrium because of the short interval of 60 to 90 minutes between

administration and the peak in uterine activity. There is little evidence for a luteolytic action in women (Karim, 1972), but  $\text{PGF}_{2\alpha}$  has been shown to be luteolytic in the cycling cow (Louis, Hafs and Morrow, 1972; Lauderdale, 1972b) and sheep (McCracken, Glew and Scaramuzzi, 1970). In the pregnant ewe detectable levels of  $\text{PGF}_{2\alpha}$  occurred only in the maternal cotyledons and increased rapidly within 24 hours after DXMS infusions to the fetus (Liggins et al., 1972). Concentrations of  $\text{PGF}_{2\alpha}$  were detectable in the myometrium and uterine vein only after labor commenced.

Challis et al. (1972) studied the temporal relationship between estrogen and  $\text{PGF}_{2\alpha}$  in the uterine venous plasma of an ovariectomized ewe at parturition. Total unconjugated estrogen levels were found to rise during the last 30 hours of pregnancy but  $\text{PGF}_{2\alpha}$  levels remained low until eight hours before lambing when their concentrations increased sharply. A similar association between estrogen and  $\text{PGF}_{2\alpha}$  in late term ewes was reported by Liggins et al., 1972.  $\text{PGF}_{2\alpha}$  infused into the aorta of pregnant sheep caused an increase in plasma estradiol-17 $\beta$  levels. He suggested the two hormones may compliment the synthesis of each other.

In the pregnant ewe PG may act primarily as a myometrial stimulant as in women. Placenta progesterone production may be influenced more by fetal estrogens or corticoid levels rather than by PG levels in the maternal placenta. This would

seem consistent with the findings of Liggins et al. (1972), Stabenfeldt, Drost and Franti (1972) and Thompson (1973) that parturition can occur in some ewes without a significant withdrawal of maternal plasma progesterone.

Recent studies in the pregnant rat have shown  $\text{PGF}_{2\alpha}$  to be an important part of parturition. As with most other species, fetal corticoids rise in the rat between 17 days and term (Milkovic, Milkovic and Paunovic, 1973) and plasma progesterone falls the last two days preceding labor (Wiest, 1968). Chester et al. (1972) was able to delay parturition in the rat by anti-inflammatory agents which inhibit the biosynthesis of PG (aspirin, indomethacin and Fenclozic acid). The administration of  $\text{PGF}_{2\alpha}$  prevented this delay.

Lauderdale (1972b) induced abortion in cows pregnant 40 to 180 days with  $\text{PGF}_{2\alpha}$ . The cows aborted in two to seven days after treatment was initiated. Delivery was slow and difficult in many of these cows. The difficult deliveries probably relate to the lower estrogen levels occurring at this stage of pregnancy. Progesterone levels were not determined but it is tempting to speculate that PG was luteolytic in these cows.

In this study the rapid (10-15 hours) depression of progesterone levels in the utero-ovarian effluent suggests a luteolytic response following DXMS treatment. Plasma estrogen levels increased slightly during this period, but did not differ significantly from pre-treatment levels. This differs

from the report of Edqvist et al. (1972) that estrogens increase sharply after DXMS treatment. In naturally calving cows, estrogens are high during the last few weeks of term, but an abrupt increase occurs only in the final 24 hours preceding labor. Plasma progesterone is already depressed by this time.

The results of this study suggest that the effectiveness of DXMS to depress plasma progesterone and induce parturition was related to plasma estrogen levels. This response did not occur unless plasma estrone levels were near 400 pg/ml. This relationship may be mediated through an estrogen dependent release of PG in small tonic levels. Caldwell et al. (1972) demonstrated a significant rise in PG levels in the peripheral plasma of ovariectomized non-pregnant ewes when estradiol administration followed prior progesterone injections. This was not evident in ovariectomized and hysterectomized ewes, or in those ovariectomized ewes treated only with progesterone. Estrogens were reported to stimulate the release of  $\text{PGF}_{2\alpha}$  from the uteri of non-pregnant guinea pigs (Blatchley et al., 1971).

Another mechanism which could be triggered by endogenous or exogenous corticosteroids may involve a placenta luteotropin. The withdrawal of such a luteotropin may be responsible for luteal regression in the cow. The possibility of a competitive



inhibition between  $\text{PGF}_{2\alpha}$  and such a luteotropin is also possible.

In keeping with this theory, HCG administration to DXMS treated cows revealed that some cows failed to have successful induction when progesterone levels failed to fall significantly. These cows had estrone levels nearly equal to those cows which induced after DXMS treatment alone (Figure 31). HCG and DXMS treated cows that calved had nearly twice the level of plasma estrone as the other cows.

Failure of HCG to block parturition in cows with high plasma estrone levels prior to treatment suggests that estrone may participate in the luteolytic mechanism or estrone may participate directly in parturition by increasing myometrial sensitivity. High estrone levels may simply indicate that parturition is near and that progesterone production sites are no longer responsive to HCG.

Those species which tolerate higher plasma progesterone levels during labor, for example women, guinea-pigs and possibly sheep, may be alternately affected by decreased protein binding sites in the myometrium. Davies and Ryan (1972) injected labelled progesterone into the rat at midpregnancy. Progesterone was taken up specifically by the myometrium at a level that exceeded that of the plasma. This uptake was markedly reduced near term. Receptor sites in the cytosol of the rat myometrium were found to decrease during the last week

of pregnancy (Davies and Ryan, 1973).

In addition the enzymatic activity of  $20\alpha$  hydroxysteroid dehydrogenase and  $5\alpha$  steroid dehydrogenase increases greatly in the uterine wall of pregnant rats after mid-term (Flint and Armstrong, 1973). Thus uterine progesterone binding is decreased and progesterone metabolism increased as parturition nears in the rat. A variation of this same mechanism may be functional in other species, particularly those that have elevated plasma progesterone levels during parturition.

It appears that DXMS induced parturition is dependent upon a complex mechanism yet to be fully understood. The mechanism undoubtedly is in delicate balance until DXMS intervention. The ultimate result is a precipitous drop in maternal plasma progesterone and parturition.

## VI. CONCLUSION

Dexamethasone (DXMS) treatment of late term pregnant cows induced parturition in about 48 hours. Maternal estrogen and progesterone levels changed in a pattern similar to levels occurring in natural parturition.

Parturition was preceded by a rise in plasma estrogens after DXMS treatment. The greatest estrogen increase occurred within the last 12-24 hours before calving. Estrone, averaging nearly 10 times the level of estradiol, was the major plasma estrogen in late pregnancy. Estrone levels increased proportionately more than estradiol as parturition neared.

Diminution of plasma progesterone was a prerequisite of premature parturition. The venous effluent from the ovary with the corpus luteum of pregnancy contained at least twice the level of progesterone present in the jugular or uterine veins. Progesterone levels fell rapidly in 15 to 24 hours after DXMS treatment and remained at low levels for 12 to 24 hours prior to calving. Pre-treatment progesterone levels were significantly higher ( $P < .01$ ) than progesterone levels at calving.

Maternal plasma corticosteroids were depressed below pre-treatment levels by DXMS. Some cows showed a rise in plasma corticosteroids at calving and others did not.

Following calving plasma estrogens dropped to very low levels for the remainder of the testing period. During the postpartum period progesterone remained at low levels, but plasma corticoids returned to normal levels by three days after calving.

Administration of human chorionic gonadotropin (HCG) prior to DXMS treatment did not increase the levels of plasma progesterone but did prevent premature parturition in two of six cows. Plasma progesterone levels did not decrease in the two cows which did not calve. This supports the proposition that maternal plasma progesterone must fall prior to parturition in the cow.

The effectiveness of DXMS to induce labor was related to maternal plasma estrogen levels prior to treatment. Cows that calved had plasma estrone levels near or above 400 pg/ml prior to DXMS treatment. Cows receiving HCG and DXMS treatments that did not calve had significantly lower ( $P < .05$ ) plasma estrone levels than calving cows. HCG may have increased the level of estrogen necessary for DXMS induction of labor or cows with lower estrogen levels may have been more responsive to HCG treatments.

Dexamethasone induced premature parturition resulted in delayed involution whenever retention of the fetal placenta occurred. Those cows which had no retention of fetal membranes involuted at the normal rate. Physical involution of the

uterus as judged by rectal palpation or necropsy was considerably delayed at two weeks postpartum but was nearly normal by four weeks after calving. Ovarian activity during the postpartum period was normal for nursed cows.

Histological examination of uterine tissue collected by biopsy or necropsy revealed endometrial resolution was delayed in treated cows with placental retention. Re-epithelization of the caruncular surface, usually complete by four weeks in normal cows, was noticeably delayed in these cows. Caruncular re-epithelization was normal in all cows by 42 days. Caruncular size and resolution (removal of devitalized tissue by histiocytes) was delayed in a similar manner. The inter-caruncular epithelium often was necrosed when acute inflammation accompanied placental retention. The epithelium was re-established by four weeks postpartum, but inflammatory cells, including neutrophils and lymphocytes, persisted in increased numbers at the six weeks postpartum period. Involution of the endometrial glands, the myometrium and the uterine vessels was unaffected in the treated cows.

In general, uterine involution was delayed in the early postpartum by 7 to 10 days and less at 42 days postpartum. Normal ovarian activity usually is not resumed in the normal cow until 35-50 days after calving. This would allow ample time for uterine involution in the DXMS treated cows. Treated

cows calved approximately 7 to 10 days early, a time lag nearly identical with the delay in uterine involution. Thus treated cows completed uterine involution at least as quickly as untreated cows of equivalent gestational age.

## VII. LITERATURE CITED

- Adams, W. M. 1969. The elective induction of labor and parturition in cattle. *Journal of the American Veterinary Medical Association* 154:261-265.
- Adams, W. M., and W. C. Wagner. 1970. The role of corticoids in parturition. *Biology of Reproduction* 3:223-227.
- Ainsworth, L., and K. J. Ryan. 1966. Steroid hormone transformation by endocrine organs from pregnant animals. I. Estrogen biosynthesis by mammalian placental preparation in vitro. *Endocrinology* 79:875-883.
- Ainsworth, L., M. Deaner, and K. J. Ryan. 1969. Steroid hormone transformation by endocrine organs for pregnant mammals. IV. Biosynthesis and metabolism of estrogens and progesterone by primate placental preparation in vitro. *Endocrinology* 84:1421-1429.
- Alexander, D. P., H. G. Britton, V. H. T. James, D. A. Nixon, R. A. Parker, E. M. Wintour, and R. D. Wright. 1968. Steroid secretion by the adrenal gland of foetal and neonatal sheep. *Journal of Endocrinology* 40:1-13.
- Allen, W. M., and S. R. M. Reynolds. 1935. Physiology of the corpus luteum. The comparative actions of crystalline progestin and crude progestin on uterine motility. *American Journal of Obstetrics and Gynecology* 30:309-318.
- Alm, C. C., J. Sullivan, and N. L. First. 1972. Dexamethasone induced parturition in the mare. *Journal of Animal Science* 35:1115. (Abstract)
- Anderson, A. B. M., C. G. Pierrepont, K. Griffiths, and A. C. Turnbull. 1972a. Steroid metabolism in the adrenals of fetal sheep in relation to natural and corticotrophin-induced parturition. *Journal of Reproduction and Fertility, Supplement* 16:25-37.
- Anderson, G. G., J. C. Hobbins, and L. Speroff. 1972b. Intravenous prostaglandins  $E_2$  and  $F_{2\alpha}$  for the induction of term labor. *American Journal of Obstetrics and Gynecology* 112:382-386.
- Arije, G. F., J. N. Wiltbank, and M. L. Hopwood. 1971. Hormone levels in pre- and post-parturient cows. *Journal of Animal Science* 1:247-248. (Abstract)

- Balfour, W. E. 1953. Changes in the hormone output of the adrenal cortex of the young calf. *Journal of Physiology* 122:59-65.
- Barr, W., and W. C. M. K. Naismith. 1972. Oral prostaglandins in the induction of labour. *British Medical Journal* 2: 188-191.
- Bassett, J. M., T. J. Oxborrow, I. D. Smith, and G. D. Thorburn. 1969. The concentration of progesterone in the peripheral plasma of the pregnant ewe. *Journal of Endocrinology* 45: 449-457.
- Bassett, J. M., and G. D. Thorburn. 1969. Foetal plasma corticosteroids and the initiation of parturition in sheep. *Journal of Endocrinology* 44:285-286.
- Beazley, J. M., and A. Gillespie. 1971. Double-blind trial of prostaglandin E<sub>2</sub> and oxytocin in induction of labour. *Lancet* 1:152-155.
- Bedford, C. A., J. R. G. Challis, F. A. Harrison and R. B. Heap. 1972. The role of oestrogens and progesterone in the onset of parturition in various species. *Journal of Reproduction and Fertility, Supplement* 16:1-23.
- Beischer, N. A., J. B. Brown, and L. Townsend. 1969. Studies in prolonged pregnancy. III. Amniocentesis in prolonged pregnancy. *American Journal of Obstetrics and Gynecology* 103:496-503.
- Bengtsson, L. P., and B. M. Schofield. 1963. Progesterone and the accomplishment of parturition in the sheep. *Journal of Reproduction and Fertility* 5:423-431.
- Beyer, C., and F. Mena. 1970. Parturition and lactogenesis in rabbits with high spinal cord transection. *Endocrinology* 87:195-197.
- Binns, W., J. F. Lynn, J. L. Shupe, and G. A. Everett. 1963. A congenital cyclopian-type malformation in lambs induced by maternal ingestion of a range plant, Veratrum californicum. *American Journal of Veterinary Research* 24:1164-1175.
- Binns, W., E. J. Thacker, L. F. James, and W. T. Huffman. 1959. A congenital cyclopian-type malformation in lambs. *Journal of American Veterinary Medical Association* 134: 180-183.



- Björkman, Nils. 1970. An atlas of placental fine structure. Alden Press, Oxford. 96 pp.
- Blatchley, F. R., B. T. Donovan, N. L. Poyser, E. W. Horton, C. J. Thompson, and M. Los. 1971. Identification of prostaglandin  $F_{2\alpha}$  in the utero-ovarian blood of guinea-pigs after treatment with oestrogen. *Nature*, London 230: 243-244.
- Blom, A. K., and O. Lyngset. 1971. Plasma progesterone levels in goats during pregnancy measured by competitive protein binding. *Acta Endocrinologica* 66:471-477.
- Bosc, M. J. 1972. The induction and synchronization of lambing with the aid of dexamethasone. *Journal of Reproduction and Fertility* 28:347-357.
- Brunner, M. A., L. E. Donaldson, and W. Hansel. 1969. Exogenous hormone and luteal function in hysterectomized and intact heifers. *Journal of Dairy Science* 52:1849-1853.
- Buch, N. C., W. J. Tyler, and L. E. Casida. 1955. Postpartum estrus and involution of the uterus in an experimental herd of Holstein-Friesian cows. *Journal of Dairy Science* 38:73-79.
- Bygdeman, M., U. Roth-Brandel, and N. Wikvist. 1970. Induction of labour by intravenous infusion of prostaglandin. *International Journal of Gynaecology and Obstetrics* 8: 162. (Abstract)
- Caldwell, B. V., S. A. Tillson, W. A. Brock, and L. Speroff. 1972. The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes. *Prostaglandins* 1:217-228.
- Cameron, N. W., and O. T. Fosgate. 1964. Effects of oxytocin upon the reproductive tract of the lactating bovine. *Journal of Dairy Science* 47:79-81.
- Casida, L. E. 1968. Studies on the postpartum cow: The postpartum cow---a resumé. *Wisconsin Agricultural Experiment Station Research Bulletin* 240:40-47.
- Casida, L. E., and W. Wisnicky. 1950. Effects of diethylstilbesterol dipropionate upon post-partum changes in the cow. *Journal of Animal Science* 9:238-243.

- Catchpole, H. R. 1969. Hormonal mechanisms during pregnancy and parturition. Pages 415-440 in H. H. Cole and P. T. Cupps, eds. Reproduction in domestic animals. 2nd Edition. Academic Press, Inc., New York.
- Challis, J. R. G. 1971. Sharp increase in free circulating oestrogens immediately before parturition in sheep. *Nature*, London 229:208.
- Challis, J. R. G., and J. L. Linzell. 1971. The concentration of total unconjugated oestrogens in the plasma of pregnant goats. *Journal of Reproduction and Fertility* 26:401-404.
- Challis, J. R. G., F. A. Harrison, and R. B. Heap. 1971a. Uterine production of estrogens and progesterone at parturition in the sheep. *Journal of Reproduction and Fertility* 25:306-307. (Abstract)
- Challis, J. R. G., R. B. Heap, and D. V. Illingworth. 1971b. Concentration of oestrogen and progesterone in the plasma of non-pregnant, pregnant and lactating guinea-pigs. *Journal of Endocrinology* 51:333-345.
- Challis, J. R. G., F. A. Harrison, R. B. Heap, E. W. Horton, and N. L. Poyser. 1972. A possible role of estrogens in the stimulation of postaglandin  $F_{2\alpha}$  output at the time of parturition in a sheep. *Journal of Reproduction and Fertility* 30:485-488.
- Chard, T. 1972. The posterior pituitary in human and animal parturition. *Journal of Reproduction and Fertility*, Supplement 16:121-138.
- Chard, T., N. R. H. Boyd, C. R. W. Edwards, and C. N. Hudson. 1971. The release of oxytocin and vasopressin by the human fetus during labour. *Nature*, London 234:352-354.
- Chard, T., N. R. H. Boyd, M. L. Forsling, A. S. McNeilly, and J. Landon. 1970. The development of a radioimmunoassay for oxytocin: The extraction of oxytocin: The extraction of oxytocin from plasma, and its measurement during parturition in human and goat blood. *Journal of Endocrinology* 48:223-234.
- Chester, R. M., S. R. Dukes, S. R. Slater, and A. L. Walpole. 1972. Delay of parturition in the rat by anti-inflammatory agents which inhibit the biosynthesis of prostaglandins (aspirin, indomethacin). *Nature*, London 240:37-38.

- ChesterJones, I., I. G. Jarret, G. P. Vinso, and K. Potter. 1964. Adrenocorticosteroid production of foetal sheep near term. *Journal of Endocrinology* 29:211-217.
- Clapp, H. 1937. A factor in the breeding efficiency in cattle. *Proceedings of American Society of Animal Production* 1937:259-264.
- Comerford, J. B. 1965. Pregnancy with anencephaly. *Lancet* 1:679-680.
- Comline, R. S., and M. Silver. 1961. The release of adrenaline and noradrenaline from the adrenal glands of the fetal sheep. *Journal of Physiology, London* 156:424-444.
- Comline, R. S., M. Silver, and I. E. Silver. 1970a. Effect of foetal hypophysectomy on catecholamine levels in the lamb adrenal during prolonged gestation. *Nature, London* 225:739-740.
- Comline, R. S., P. W. Nathanielsz, R. B. Paisley, and M. Silver. 1970b. Cortisol turnover in the sheep foetus immediately prior to parturition. *Journal of Physiology, London* 210:141-142.
- Csapo, A. 1956. Progesterone "block". *American Journal of Anatomy* 98:273-291.
- Csapo, A. 1969. The four direct regulatory factors of myometrial function. Pages 13-42 in G. E. W. Wolstenholme, and J. Knight eds. *Progesterone: Its regulatory effect on the myometrium*. Ciba Foundation Study Group No. 34. J. and A. Churchill, London.
- Csapo, A., and J. P. Sauvage. 1968. The evolution of uterine activity during human pregnancy. *Acta Obstetricia Et Gynecologica Scandinavica* 47:181-212.
- Csapo, A., and H. Takeda. 1965. Effects of progesterone on the electric activity and intrauterine pressure or pregnant and parturient rabbits. *American Journal of Obstetrics and Gynecology* 91:221-227.
- Csapo, A., and W. G. Wiest. 1969. An examination of the quantitative relationship between progesterone and the maintenance of pregnancy. *Endocrinology* 85:735-746.

- Csapo, A., J. P. Sauvage, and W. G. Wiest. 1971a. The efficacy and acceptability of intravenously administered prostaglandins for an abortifacient. *American Journal of Obstetrics and Gynecology* 111:1059-1063.
- Csapo, A., I. E. Knobil, H. I. Van der Molen, and W. G. Wiest. 1971b. Peripheral plasma progesterone levels during human pregnancy and labor. *American Journal of Obstetrics and Gynecology* 110:630-632.
- Danforth, D. N. 1966. *Textbook of Obstetrics and Gynecology*. Hoeber, Harper, and Row, New York, New York. 465 pp.
- Davies, I. J., and K. J. Ryan. 1972. The uptake of progesterone by the uterus of the pregnant rat in vivo and its relationship to cytoplasmic progesterone-binding protein. *Endocrinology* 90:507-515.
- Davies, I. J., and K. J. Ryan. 1973. The modulation of progesterone concentration in the myometrium of the pregnant rat by changes in cytoplasmic "receptor" protein activity. *Endocrinology* 92:394-401.
- Davies, I. J., K. J. Ryan, and Z. Petro. 1970. Estrogen synthesis by adrenal placental tissues of the sheep and rhesus monkey in vitro. *Journal of Endocrinology* 86:1457-1459.
- Diczfalusy, E. R. 1964. Endocrine functions of the human fetoplacental unit. *Federation Proceedings* 23:791-798.
- Dixon, R., A. Hyman, E. Gurpide, I. Dyrenfurth, H. Cohen, E. Bowe, T. Engel, S. Daniel, S. James, and R. Vande Wiele. 1970. Feto-maternal transfer and production of cortisol in the sheep. *Steroids* 12:771-789.
- Doe, R. P., R. Fernandez, and V. S. Seal. 1964. Measurement of corticosteroid binding globulin in man. *Journal of Clinical Endocrinology* 24:1029-1039.
- Donaldson, L. E., J. B. Bassett, and G. D. Thorburn. 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrus cycles, pregnancy and lactation, and the effects of under-nutrition, or exogenous oxytocin on progesterone concentration. *Journal of Endocrinology* 48:599-614.
- Drost, M. 1972. Failure to induce parturition in pony mares with dexamethasone. *Journal of American Veterinary Association* 160:321-322.

- Drost, M., and L. W. Holm. 1968. Prolonged gestation in ewes after foetal adrenalectomy. *Journal of Endocrinology* 40: 293-296.
- Eberhart, R. J., and J. A. Patt, Jr. 1971. Plasma cortisol concentrations in newborn calves. *American Journal of Veterinary Research* 32:1921-1927.
- Edqvist, L-E., L. Ekman, B. Gustafsson, S-O. Jacobson, E. D. B. Johansson, and J. O. Lindell. 1972. Peripheral plasma levels of oestrone and progesterone in pregnant cows treated with dexamethasone. *Acta Endocrinologica* 71: 731-742.
- Edqvist, L-E., L. Ekman, B. Gustafsson, and E. D. B. Johansson. 1973. Peripheral plasma levels of estrogens and progesterone during late bovine pregnancy. *Acta Endocrinologica* 72:81-88.
- Edwards, M. J. 1950. Fertility in cattle: Effect of postpartum interval. *Veterinary Record* 62:310-311.
- Edwards, M. J. 1962. Weights of cyclic and pregnancy corpora lutea of dairy cows. *Journal of Reproduction and Fertility* 4:93-98.
- Embrey, M. P., and K. Hillier. 1971. Therapeutic abortion by intrauterine instillation of prostaglandins. *British Medical Journal* 1:588-590.
- Erb, R. E., V. L. Estergreen, R., W. R. Gomes, E. D. Plotka, and O. L. Frost. 1968. Progestin levels in corpora lutea and progesterone in ovarian venous and jugular vein blood plasma of the pregnant bovine. *Journal of Dairy Science* 51:401-409.
- Erb, R. E., W. R. Gomes, R. D. Randel, V. L. Estergreen, Jr., and O. L. Frost. 1968. Effect of ovariectomy on concentration of progesterone in blood plasma and urinary estrogen excretion rate in the pregnant bovine. *Journal of Dairy Science* 51:420-427.
- Estergreen, V. L., Jr., O. L. Frost, W. R. Gomes, R. E. Erb, and J. F. Bullard. 1967. Effect of ovariectomy on pregnancy maintenance and parturition on dairy cows. *Journal of Dairy Science* 50:1293-1295.
- Findlay, J. K., and R. F. Seamark. 1971. The biosynthesis of oestrogens in the ovine foeto-placental unit. *Journal of Reproduction and Fertility* 24:141-147.

- First, N. L., and R. B. Stagmiller. 1972. The effects of ovariectomy, dexamethasone, and progesterone on the maintenance of pregnancy in swine. *Journal of Animal Science* 35:1118-1119. (Abstract)
- Fitzpatrick, R. J., and C. F. Walmsley. 1965. The release of oxytocin during parturition. Pages 51-71 in J. H. M. Pinkerton. ed. *Advances in oxytocin research*. Pergamon Press, New York.
- Flint, A. P. F., and D. T. Armstrong. 1973. The appearance of an endometrial 20 $\alpha$ -hydroxysteroid dehydrogenase towards the end of pregnancy in the rat. *Endocrinology* 92:624-627.
- Foote, W. D., and J. E. Hunter. 1964. Post-partum intervals of beef cows treated with progesterone and estrogen. *Journal of Animal Science* 23:517-520.
- Foote, W. D., E. R. Hauser, and L. E. Casida. 1960. Some causes of variation in post-partum reproductive activity in Hereford cows. *Journal of Animal Science* 19:238-241.
- Foote, W. D., D. C. Sawhney, M. M. Quevedo, and D. W. Peterson. 1966. Chorionic gonadotropin treatment in beef cows. *Journal of Animal Science* 25:585-586. (Abstract)
- Fuchs, A-R. 1964. Oxytocin and the onset of labour in rabbits. *Journal of Endocrinology* 30:217-224.
- Fuchs, A-R., and V. F. Poblete, Jr. 1970. Oxytocin and uterine function in pregnant and parturient rats. *Biology of Reproduction* 2:387-400.
- Fuchs, F., A-R. Fuchs, and R. V. Short. 1963. Human placental progesterone-source at pregnancy. *Journal of Endocrinology* 27:333-339.
- Fylling, P. 1971. Premature parturition following dexamethasone administration to pregnant ewes. *Acta Endocrinologica* 66:289-295.
- Gier, H. T., and G. B. Marion. 1968. Uterus of the cow after parturition: Involutional changes. *American Journal of Veterinary Research* 29:83-96.
- Gier, H. T., N. P. Singh, and G. B. Marion. 1962. Histo-pathology of the postpartum bovine uterus. *Journal of Animal Science* 21:1023-1035.

- Gronborg-Pedersen, H. 1969. Drug induced calving. *Nordis Veterinarmedicin* 21:591-597.
- Grota, L. J., and K. B. Eik-Nes. 1967. Plasma progesterone concentrations during pregnancy and lactation in the rat. *Journal of Reproduction and Fertility* 13:83-91.
- Hagino, N. 1972. The effect of synthetic corticosteroids on ovarian function in the baboon. *Journal of Clinical Endocrinology and Metabolism* 35:716-721.
- Hammond, J. 1929. The physiology of reproduction in the cow. Cambridge Press, London. 226 pp.
- Hansen, L. H., and J. Christiansen. 1971. Undersogelse af den fodselsinducerende virkning hoskvaeg af et corticosteroid (dexamethason). Induction of parturition in cows with a corticosteroid. *Nordisk Veterinarmedicin* 23:162-169.
- Harvey, W. R. 1960. Least-squares analysis of data with unequal subclass numbers. U.S. Department of Agriculture ARS-20-8. 15 pp.
- Heap, R. B., and R. Deanesly. 1966. Progesterone in systemic blood and placenta of intact and ovariectomized pregnant guinea-pigs. *Journal of Endocrinology* 34:417-423.
- Heckel, G. P., and W. M. Allen. 1939. Maintenance of the corpus luteum and inhibition of parturition in the rabbit by injection of oestrogenic hormone. *Endocrinology* 24:137-148.
- Henricks, D. M., J. F. Dickey, J. R. Hill, and W. E. Johnston. 1972. Plasma estrogen and progesterone levels after mating and during late pregnancy and postpartum in cows. *Endocrinology* 90:1336-1342.
- Hindson, J. C., B. M. Schofield, and C. B. Turner. 1967. The effect of a single dose of stilbesterol on cervical dilatation in pregnant sheep. *Research in Veterinary Science* 8:353-360.
- Hindson, J. C., B. M. Schofield, and W. R. Ward. 1969. The effect of progesterone on recorded parturition and on oxytocin sensitivity in the sheep. *Journal of Endocrinology* 43:207-215.
- Hoag, R. W. 1971. Use of urinary estriol determinations in high-risk pregnancy in the community hospital. *Journal of Obstetrics and Gynecology* 110:203-209.

- Holm, L. W., and R. V. Short. 1962. Progesterone in the peripheral blood of Guernsey and Friesian cows during prolonged gestation. *Journal of Reproduction and Fertility* 4:137-141.
- Holm, L. W., H. R. Parker, and S. J. Galligan. 1961. Adrenal insufficiency in postmature Holstein calves. *American Journal of Obstetrics and Gynecology* 8:1000-1008.
- Holmdahl, T. H., E. D. B. Johansson, and B. A. Nilsson. 1971. Plasma progesterone in pregnancy interrupted by the intra-uterine injection of hypertonic saline. *Acta Endocrinologica* 66:82-88.
- Hunter, D. L., R. E. Erb, R. C. Randel, H. A. Farverick, C. J. Callahan, and R. B. Harrington. 1970. Reproductive steroids in the bovine. I. Relationships during late gestation. *Journal of Animal Science* 30:47-51.
- Illingworth, D. V., R. B. Heap, and J. S. Perry. 1970. Changes in the metabolic clearance rate of progesterone in the guinea-pig. *Journal of Endocrinology* 48:409-417.
- Jackson, B. T., and G. J. Piasecki. 1969. Fetal secretion of glucocorticoids. *Endocrinology* 85:875-880.
- Jöchle, W., H. Esparza, T. Gimenez, and M. A. Hidalgo. 1972. Inhibition of corticoid-induced parturition by progesterone in cattle: Effect on delivery and calf viability. *Journal of Reproduction and Fertility* 28:407-412.
- Karim, S. M. M. 1968. Appearance of prostaglandin  $F_{2\alpha}$  in human blood during labour. *British Medical Journal* 4: 618-619.
- Karim, S. M. M. 1972. Physiological role of prostaglandins in the control of parturition and menstruation. *Journal of Reproduction and Fertility*, Supplement 16:105-119.
- Kempthorne, O. 1952. Analysis of multiple classification. Pages 68-119 in O. Kempthorne, ed. *Design and analysis of experiments*. John Wiley and Son Inc., New York, New York.
- Kendall, J. Z., and G. C. Liggins. 1972. The effect of dexamethasone on pregnancy in the rabbit. *Journal of Reproduction and Fertility* 29:409-413.



- Kennedy, P. C., J. W. Kendrick, and C. Stormont. 1957. Adenohypophyseal aplasia, an inherited defect associated with abnormal gestation in Guernsey cattle. *Cornell Veterinarian* 47:160-178.
- Killian, D. B., H. A. Garnerick, and B. N. Day. 1972. Peripheral plasma progesterone and corticoid levels at parturition in the sow. *Journal of Animal Science* 35: 1119-1125.
- Kim, M. H., R. Borth, P. H. McCleary, C. A. Woolever, and P. C. M. Young. 1971. Sex hormone secretion of the placenta left in situ after ovarian pregnancy. *American Journal of Obstetrics and Gynecology* 110:658-662.
- Kimura, G., and W. S. Cornwell. 1938. The progestin contents of the corpus luteum of the sow (*Sus scrofa*) during successive stages of the estrous cycle and pregnancy. *American Journal of Physiology* 123:471-476.
- Korenman, S. G., D. Tulchinsky, and L. W. Eaton. 1970. Radioligand procedures for estrogen assay in normal and pregnancy plasma. Pages 291-301 in E. Diczfalussy, ed. *Steroid assay by protein-binding*. Bogtrykkeriet Forum, Copenhagen.
- Kudlac, E., and B. Benysek. 1969. Effects of corticosteroids on blood picture in cows after parturition and their following fertility. *Acta Veterinaria* 38:413-422.
- Labhsetwar, A. P. 1972. Luteolytic and ovulation-inducing properties of prostaglandin  $F_{2\alpha}$  in pregnant mice. *Journal of Reproduction and Fertility* 28:451-452.
- Labhsetwar, A. P., W. E. Collins, W. J. Tyler, and L. E. Casida. 1964. Some pituitary ovarian relationships in peri-parturient cow. *Journal of Reproduction and Fertility* 8:85-90.
- Lauderdale, J. W. 1972a. Effect of corticoid administration on bovine pregnancy. *Journal of American Veterinary Medical Association* 160:867-871.
- Lauderdale, J. W. 1972b. Effects of  $PGF_{2\alpha}$  on pregnancy and estrous cycle of cattle. *Journal of Animal Science* 35: 246.

- Lehmann, F., F. Peters, M. Breckwoldt, and G. Bettendorf. 1972. Plasma progesterone levels during infusion of prostaglandin in  $F_{2\alpha}$  in the human. Prostaglandins 1: 269-277.
- LeMaire, W. J., W. N. Spellacy, A. B. Shevach, and S. A. Gall. 1972. Changes in plasma estriol and progesterone during labor induced with prostaglandin  $F_{2\alpha}$  or oxytocin. Prostaglandins 1:93-101.
- Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effects of prostaglandin on cattle estrus cycles. Journal of Animal Science 35:247. (Abstract)
- Liggins, G. C. 1968. Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. Journal of Endocrinology 42:323-329.
- Liggins, G. C. 1969a. Premature delivery of foetal lambs infused with glucocorticoids. Journal of Endocrinology 45:515-523.
- Liggins, G. C. 1969b. Foetal role in the initiation of parturition in the ewe. Pages 218-230 in G. E. W. Wolstenholme and M. O'Connor, eds. Fetal autonomy. Ciba Foundation Symposium. J. and A. Churchill, London.
- Liggins, G. C., and Susan Grieves. 1971. Possible role for prostaglandin  $F_{2\alpha}$  in parturition in sheep. Nature, London 232:629-631.
- Liggins, G. C., P. C. Kennedy, and L. W. Holm. 1967. Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. American Journal of Obstetrics and Gynecology 98:1080-1086.
- Liggins, G. C., S. A. Grieves, J. Z. Kendall, and B. S. Knox. 1972. The physiological roles of progesterone, oestradiol-17 $\beta$  and prostaglandin  $F_{2\alpha}$  in the control of ovine parturition. Journal of Reproduction and Fertility, Supplement 16:85-103.
- Lindner, H. R. 1964. Comparative aspects of cortisol transport: Lack of firm binding to plasma proteins in domestic ruminants. Journal of Endocrinology 28:301-320.

- Linzell, J. L., and R. B. Heap. 1968. A comparison of progesterone metabolism in the pregnant sheep and goat: Sources of production and an estimation of uptake by some target organs. *Journal of Endocrinology* 41:433-438.
- Liptrap, R. M. and J. E. Raeside. 1968. Effect of corticotrophin and corticosteroids on plasma interstitial cell-stimulating hormone and urinary steroids in the boar. *Journal of Endocrinology* 42:33-39.
- Llauro, J. L., B. Runnebaum, and J. Zander. 1968. Progesterone in human peripheral blood before, during, and after labor. *American Journal of Obstetrics and Gynecology* 101:867.
- Louis, T. M., H. D. Hafs, and D. A. Morrow. 1972. Estrus and ovulation after uterine PGF<sub>2α</sub> in cows. *Journal of Animal Science* 35:274-275. (Abstract)
- McCracken, J. A., M. E. Glew, and R. J. Scaramuzzi. 1970. Corpus luteum regression induced by prostaglandin F<sub>2α</sub>. *Journal of Clinical Endocrinology* 30:544-549.
- McDonald, L. E., S. H. McNutt, and R. E. Nichols. 1953. The essentiality of the bovine corpus luteum of pregnancy. *American Journal of Veterinary Research* 14:539-541.
- March, J. 1971. The effect of prostaglandins on the adenyl cyclase of the bovine corpus luteum. *Annals of the New York Academy of Science* 180:416-425.
- Marion, G. B., J. S. Norwood, and H. T. Gier. 1968. Uterus of the cow after parturition: Factors affecting regression. *American Journal of Veterinary Research* 29:71-75.
- Marshall, F. H. A., and J. Chassar Moir. 1952. Parturition. Pages 505-506 in A. S. Parkes, ed. *Marshall's physiology of reproduction*. Vol. II. 3rd Edition. Longmans, Green and Company, London.
- Mattner, P. E., and G. D. Thorburn. 1971. Progesterone in utero-ovarian venous plasma during pregnancy in ewes. *Journal of Reproduction and Fertility* 24:140-141.
- Melampy, R. M., W. R. Hearn, and J. M. Raker. 1959. Progesterone content of bovine reproductive organs and blood during pregnancy. *Journal of Animal Science* 18:307-316.

- Mellin, T. N., R. E. Erb, and V. L. Estergreen, Jr. 1966. Urinary excretion of estrogen by the bovine before and after parturition. *Journal of Animal Science* 25:955-961.
- Menge, A. C., S. E. Mares, W. J. Tyler, and L. E. Casida. 1962. Variation and association among postpartum reproductive and production characteristics in Holstein-Freisian cattle. *Journal of Dairy Science* 45:233-244.
- Milgrom, E., and E. E. Baulieu. 1970. Progesterone in the uterus and the plasma. I. Binding in rat uterus 105,000 g of supernatant. *Endocrinology* 87:276-287.
- Milkovic, S., K. Milkovic, and J. Paunovic. 1973. The initiation of fetal adrenocorticotrophic activity in the rat. *Endocrinology* 92:380-384.
- Mills, R. C., and M. C. Morrisette. 1970. Progesterone synthesis by perfused bovine ovaries of early and late pregnancy. *Journal of Reproduction and Fertility* 22:435-439.
- Molokwu, E. C. I., and W. C. Wagner. 1973. Endocrine physiology of the puerperal sow. *Journal of Animal Science* 36:1158-1163.
- Morrow, D. A., S. J. Roberts, and K. McEntee. 1969. A review of postpartum ovarian activity and involution of the uterus and cervix of cattle. *Cornell Veterinarian* 59:134-154.
- Morrow, D. A., S. J. Roberts, K. McEntee and H. G. Gray. 1966. Postpartum ovarian activity and uterine involution in dairy cattle. *Journal of American Veterinary Medical Association* 149:1596-1609.
- Munson, A. K., J. R. Mueller, and M. E. Yannone. 1970. Free plasma 17 $\beta$ -estradiol in normal pregnancy, labor and the puerperium. *American Journal of Obstetrics and Gynecology* 108:340-344.
- Murphy, B. E. P. 1967. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *Journal of Clinical Endocrinology and Metabolism* 27:973-990.
- Murphy, B. E. P. 1973. Does the human fetal adrenal play a role in parturition? *American Journal of Obstetrics and Gynecology* 115:521-525.

- Nathanielsz, P. W., R. S. Comline, M. Silver, and R. B. Paisey. 1972. Cortisol metabolism in the fetal and neonatal sheep. *Journal of Reproduction and Fertility*, Supplement 16:39-59.
- Neil, J. C., E. D. B. Johansson, J. K. Datta, and E. Knobil. 1967. Relationship between the plasma levels of luteinizing hormone and progesterone during normal menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* 27:1167-1173.
- Nelson, D. W., and E. P. Smith. 1963. Urinary Excretion of estrogenic compounds during estrus and gestation by bovine as determined by three assay methods. *Journal of Dairy Science* 46:135-139.
- North, S. A., E. R. Hauser, and N. L. First. 1973. Induction of parturition in swine and rabbits with the corticosteroid dexamethasone. *Journal of Animal Science* 36:1170-1174.
- Osinga, A., T. H. Stegenga, and W. Jöchle. 1971. Initiation of parturition of dairy cows with a synthetic corticosteroid. *Sonderdruck Aus Zuchthygiene* 5:64-69. (English Reprint)
- Oxender, W. D., H. D. Hafs, and L. A. Edgerton. 1972. Serum growth hormone, LH, and prolactin in the pregnant cow. *Journal of Animal Science* 35:51-55.
- Oxenreider, S. L. 1968. Effects of suckling and ovarian function on postpartum reproductive activity in beef cows. *American Journal of Veterinary Research* 29:2099-2102.
- Oxenreider, S. L., and W. C. Wagner. 1971. Postpartum ovarian activity. *Journal of Animal Science* 33:1026-1031.
- Paisey, R. B., and P. W. Nathanielsz. 1971. Plasma cortisol levels in the newborn lamb from birth to 30 days. *Journal of Endocrinology* 50:701-702.
- Paterson, J. Y. F., and F. Hills. 1967. The binding of cortisol by ovine plasma proteins. *Journal of Endocrinology* 37:261-268.
- Perkins, J. L., and H. E. Kidder. 1963. Relation of uterine involution and postpartum interval to reproductive efficiency in beef cattle. *Journal of Animal Science* 22:313-315.

- Pharriss, B. B., S. A. Tillson, and R. R. Erickson. 1972. Prostaglandins in luteal function. Pages 51-89 in E. B. Astwood, ed. Recent Progress in Hormone Research. Academic Press, New York.
- Pharriss, B. B., and L. J. Wyngarden. 1969. The effects of prostaglandin  $F_{2\alpha}$  on the progesterone content of ovaries from pseudopregnant rats. Proceedings of the Society for Experimental Biology and Medicine 130:92-94.
- Pierrepont, C. G., A. B. M. Anderson, G. Harvey, A. C. Turnbull, and K. Griffiths. 1971. The conversion in vitro of  $C_{19}$ -steroids to oestrogen sulphates by the sheep placenta. Journal of Endocrinology 50:537-541.
- Pope, G. S., S. K. Gupta, and I. B. Munro. 1969. Progesterone levels in the systemic plasma of pregnant, cycling, and ovariectomized cows. Journal of Reproduction and Fertility 20:369-381.
- Pope, G. S., H. E. H. Jones, and H. B. Waynforth. 1965. Oestrogens in the blood of the cow. Journal of Endocrinology 33:385-395.
- Porter, D. G. 1970. The failure of progesterone to affect myometrial activity in the guinea-pig. Journal of Endocrinology 46:425-434.
- Porter, D. G., and H. R. Behrman. 1971. Prostaglandin-induced myometrial activity inhibited by progesterone. Nature, London 232:627.
- Randel, R. D., and R. E. Erb. 1971. Reproductive steroids in the bovine. VI. Changes and inter-relationships from 0 to 260 days of pregnancy. Journal of Animal Science 31:115-123.
- Rasbech, N. O. 1950. The normal involution of the uterus in the cow. Nordish Veterinarmedicin 2:655-687.
- Rea, C. 1898. Prolonged gestation, acrania monstrosity and apparent placenta praevia in one obstetrical case. Journal of the American Medical Association 30:1116-1167.
- Reynolds, R. S. M. 1959. Gestation mechanism. Annals of the New York Academy of Sciences 75:691-714.

- Rich, T. P., G. W. Libal, L. R. Dunn, and R. C. Wahlstrom. 1972. Induction of parturition in sows with dexamethasone. *Journal of Animal Science* 35:1120. (Abstract)
- Riesen, J. W., S. Saiduddin, W. J. Tyler, and L. E. Casida. 1968. Relation of postpartum interval to corpus luteum development, pituitary prolactin activity, and uterine involution in dairy cows. *Wisconsin Agricultural Experiment Station Research Bulletin* 270:27-40.
- Roberts, J. S., and L. Share. 1969. Effects of progesterone and estrogens on blood levels of oxytocin during vaginal distention. *Endocrinology* 84:1076-1081.
- Roberts, S. J. 1971. IV. Physiology of the gestation period. Pages 81-106 in S. J. Roberts ed. *Veterinary Obstetrics and Genital Disease*. 2nd edition. Edward Brothers Inc., Ann Arbor, Michigan.
- Robinson, R., P. A. Anastassiadis, and R. H. Common. 1971. Estrone concentrations in the peripheral blood of pregnant cows. II. Values around parturition. *Journal of Dairy Science* 54:1832-1839.
- Rosenthal, H. E., W. R. Slaunwhite, Jr., and A. A. Sandberg. 1969. Transcortin: A corticosteroid-binding protein of plasma. X. Cortisol and progesterone interplay and unbound level of these steroids in pregnancy. *Journal of Clinical Endocrinology* 29:352-367.
- Ryan, K. J. 1969. Theoretical basis for endocrine control of gestation--A comparative approach. Page 120 in A. Pecile and C. Finzi, eds. *The feto-placental unit*. *Excerpta Medica*, Amsterdam.
- Ryan, K. J. 1971. Endocrine control of gestational length. *American Journal of Obstetrics and Gynecology* 100:299-306.
- Saiduddin, S. S., J. W. Riesen, W. J. Tyler, and L. E. Casida. 1968. Relation of postpartum interval to pituitary gonadotropins, ovarian follicular development and fertility in dairy cows (Effect of suckling, feeding level, breeding management and genetic level of milk production). *Wisconsin Agricultural Experiment Station Research Bulletin* 270:15-26.
- Sawhney, D. S., M. M. Quevedo and W. D. Foote. 1966. Uterine histology of cows spayed postpartum. *Journal of Animal Science* 25:584-585.

- Schams, D., B. Hoffmann, S. Fischer, E. Marz, and H. Karg. 1972. Simultaneous determination of L.H. and progesterone in peripheral bovine blood during pregnancy, normal and corticoid-induced parturition and the postpartum period. *Journal of Reproduction and Fertility* 29: 37-48.
- Seal, U. S., and R. P. Doe. 1966. Corticosteroid-binding globulin: Biochemistry physiology and phylogeny. Pages 63-90 in T. Napao, G. Pincus, and J. F. Tait, eds. *Steroid Dynamics*. Academic Press, New York.
- Shannon, F. P., G. W. Salisbury, and N. L. Van Demark. 1952. The fertility of cows inseminated at various intervals after calving. *Journal of Animal Science* 11:355-360.
- Short, R. E., R. A. Bellows, E. L. Moody, and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine post partum reproduction. *Journal of Animal Science* 34:71-74.
- Short, R. V. 1958. Progesterone in blood. II. Progesterone in the peripheral blood of pregnant cows. *Journal of Endocrinology* 16:426-434.
- Short, R. V. 1960. Blood progesterone levels in relation to parturition. *Journal of Reproduction and Fertility* 1: 61-70.
- Short, R. V. 1961. Progesterone. Pages 379-437 in C. H. Gray and A. L. Bacharach, eds. *Hormones in blood*. Academic Press, New York.
- Short, R. V., and N. W. Moore. 1959. Progesterone in blood. V. Progesterone and  $20_{\alpha}$ -hydroxypregn-4-en-3-one in the placenta and blood of ewes. *Journal of Endocrinology* 19: 288-293.
- Short, R. V., G. Wagner, A. R. Fuchs, and F. Fuchs. 1965. Progesterone concentrations in uterine venous blood after intra-amniotic injections of hypertonic saline in mid-pregnancy. *American Journal of Obstetrics and Gynecology* 19:132-135.
- Siiteri, P. K., and P. C. MacDonald. 1966. Placental estrogen biosynthesis during human pregnancy. *Journal of Clinical Endocrinology and Metabolism* 26:751-761.
- Skinner, J. D., W. Jöchle, and J. W. Nel. 1970. Induction of parturition in Karakul and crossbred ewes with flumethasone. *Agroanimalia* 2:99-100.



- Smith, V. G., L. A. Edgerton, H. D. Hafs, and E. M. Convey. 1973. Bovine serum estrogens, progestins, and glucocorticoids during late pregnancy. *Journal of Animal Science* 36:391-395.
- Smith, I. D., and R. P. Shearman. 1972. The relationship of human umbilical arterial and venous plasma levels of corticosteroids to gestational age. *Journal of Endocrinology* 55:211-212.
- Solomon, S. 1966. Formation and metabolism of neutral steroids in the human placenta and fetus. *Journal of Clinical Endocrinology and Metabolism* 26:762-772.
- Speroff, L., B. V. Caldwell, W. A. Brock, G. G. Anderson, and J. C. Hobbins. 1972. Hormone levels during prostaglandin  $F_{2\alpha}$  infusions for therapeutic abortions. *Journal of Clinical Endocrinology and Metabolism* 34:531-536.
- Speroff, L., and P. W. Ramwell. 1970. Prostaglandin stimulation of *in vitro* progesterone synthesis. *Journal of Clinical Endocrinology and Metabolism* 30:345-350.
- Stabenfeldt, G. H., M. Drost, and C. E. Franti. 1972. Peripheral plasma progesterone levels in the ewe during pregnancy and parturition. *Endocrinology* 90:144-150.
- Stabenfeldt, G. H., B. I. Osburn, and L. L. Ewing. 1970. Peripheral plasma progesterone levels in the cow during pregnancy and parturition. *American Journal of Physiology* 218:571-575.
- Stormshak, F., and R. E. Erb. 1961. Progestins in bovine corpora lutea, ovaries, and adrenals during pregnancy. *Journal of Animal Science* 44:310-320.
- Tennant, B., Kendrick, J. W., and R. G. Petticord. 1967. Uterine involution and ovarian function in the postpartum cow: A retrospective analysis of 2338 genital organ examinations. *Cornell Veterinarian* 57:543-545.
- Thompson, F. N., Jr. 1973. Feto-placental steroids and parturition in the ewe. Unpublished Ph.D. Thesis, Iowa State University, Ames, Ia. 178 pp.

- Thorburn, G. D., and D. H. Nicol. 1971. Regression of the ovine corpus luteum following infusion of prostaglandin  $F_{2\alpha}$  into the ovarian artery and uterine vein. *Journal of Endocrinology* 51:785-786.
- Thorburn, G. D., and W. Schneider. 1971. The progesterone concentration in the plasma of the goat during the oestrus cycle and pregnancy. *Journal of Endocrinology* 52:23-36.
- Thorburn, G. D., D. H. Nicol, J. M. Bassett, D. A. Shutt, and R. I. Cox. 1972. Parturition in the goat and sheep: Changes in corticosteroids, progesterone oestrogens and prostaglandin F. *Journal of Reproduction and Fertility*, Supplement 16:61-84.
- Tulchinsky, D., C. J. Hobel, and S. G. Korenman. 1971. A radioligand assay for plasma unconjugated estriol in normal and abnormal pregnancies. *American Journal of Obstetrics and Gynecology* 111:311-318.
- Tulchinsky, D., C. J. Hobel, E. Yeager, and J. R. Marshall. 1972. Plasma estrone, estradiol, estrol, progesterone, and 17-hydroxyprogesterone in human pregnancy. *American Journal of Obstetrics and Gynecology* 112:1095-1100.
- Uren, A. W. 1935. Involution of the uterine mucosa in the ewe. Michigan State Agriculture Experiment Station Technical Bulletin No. 144.
- Van Denmark, N. L., and G. W. Salisbury. 1950. The relation of the post-partum breeding interval to reproductive efficiency in the dairy cow. *Journal of Animal Science* 9:307-313.
- Van Rensburg, S. J. 1967. Gestation in sheep after foetal adrenalectomy and cortisol acetate administration. *Journal of Endocrinology* 38:83-84.
- Van Rensburg, S. J. 1971. Reproductive physiology and endocrinology of normal and habitually aborting angora goats. *Onderstepoort Journal of Veterinary Research* 38:1-62.
- Vorherr, H. 1968. Process of labor puerperium and lactation. Pages 426-448 in *Biology of Gestation*. Vol. 1. N. S. Assali, ed. Academic Press, New York.

- Wagner, W. C., and W. Hansel. 1969. Reproductive physiology of the postpartum cow. I. Clinical and histological finding. *Journal of Reproduction and Fertility* 18:493-500.
- Wagner, W. C. and S. L. Oxenreider. 1971. Endocrine physiology following parturition. *Journal of Animal Science, Supplement I*, 33:1-13.
- Wagner, W. C., R. L. Willham, and L. E. Evans. 1971. Induced parturition in the beef cow. *Journal of Animal Science* 33:1164. (Abstract)
- Wagner, W. C., R. E. Strohbehn and P. A. Harris. 1972. ACTH, Corticoids and luteal function in heifers. *Journal of Animal Science* 35:789-793.
- Warnick, A. C. 1955. Factors associated with the interval from parturition to first estrus in beef cattle. *Journal of Animal Science* 14:1003-1010.
- Whipp, S. C., and N. C. Lyon. 1970. Hydrocortisone determination in bovine plasma. *Cornell Veterinarian* 60:582-589.
- Wiest, W. G. 1968. On the function of  $20\alpha$ -hydroxypregn-4-en-3-one during parturition in the rat. *Endocrinology* 83:1181-1184.
- Wiest, W. G. 1970. Progesterone and  $20\alpha$ -hydroxypregn-4-en-3-one in plasma, ovaries and uteri during pregnancy in the rat. *Endocrinology* 87:43-48.
- Wiest, W. G., M. O. Pulkkinen, J. Sauvage, and A. I. Csapo. 1970. Plasma progesterone levels during saline-induced abortions. *Journal of Clinical Endocrinology and Metabolism* 30:774-777.
- Wiltbank, J. N., and A. C. Cook. 1958. The comparative reproductive performance of nursed and milked cows. *Journal of Animal Science* 17:640-648.
- Wright, J. M., I. Settergren, R. R. Saatman, and W. Hansel. 1970. Peripheral plasma progesterone levels in pregnant cows treated with flumethasone. *Society for the Study of Reproduction, 3rd Annual Meeting, Columbus, Ohio.* 1970:32. (Abstract)

- Yannone, M. E., J. R. McCurdy, and A. Goldfien. 1968. Plasma progesterone levels in normal pregnancy, labor and the puerperium. *American Journal of Obstetrics and Gynecology* 101:1058-1061.
- Zarrow, M. X., N. C. Anderson, and M. R. Callantine. 1963. Failure of progestogens to prolong pregnancy in the guinea-pig. *Nature, London* 198:690.

## VIII. ACKNOWLEDGEMENTS

I wish to express my appreciation to all those who have assisted my endeavor in this project. I am particularly grateful to Dr. William C. Wagner and Dr. Wallace M. Wass, who served as major professors and for their encouragement and guidance; to Dr. Richard L. Willham for statistical consultation and for providing a portion of the research animals; to the other members of my special committee, Dr. Frank Ramsey and Dr. John P. Kluge for their guidance in pathology.

The invaluable technical assistance of Miss Patsy A. Harris and Mr. R. E. Strohbehn is truly appreciated. I wish to thank the Veterinary Medical Research Institute for furnishing equipment and facilities used in this study. I am grateful to Mrs. Maxine Bogue for her assistance in the preparation and typing of this manuscript.

Finally I wish to recognize and thank my wife and children for their patience and encouragement during this period.

IX. APPENDIX

Appendix Table 1. Corticoid assay procedure

- 
1. Place 0.5 ml of plasma for each unknown into a 16 x 125 mm screw cap disposable glass tube. An equal volume of each cortisol standard (3,6,12,24,36,48 and 60 ml/ml prepared in distilled water) was placed in separate screw cap tubes.
  2. Add 4.5 ml of Nanograde methylene chloride<sup>a</sup> to each tube.
  3. Shake the tubes manually for 30 seconds.
  4. Centrifuge the extraction tubes for 10 minutes at 2000 rpm.
  5. Aspirate the upper water or plasma phase off the methylene chloride with a Pasteur pipet attached to a water syphon.
  6. Place 1 ml of the methylene chloride extract into each of two 12 x 75 mm disposable glass tubes for each unknown and into three tubes for each standard.
  7. Add one set of standard tubes to each rack of unknowns.
  8. Evaporate the methylene chloride in a 45 C water bath.
  9. To each tube add 1 ml of corticoid binding globulin (3% CBG) -<sup>3</sup>H-cortisol solution (Use an automatic refilling syringe).

The CBG was prepared as follows:

- a. Evaporate 4 ml of stock solution (<sup>3</sup>H-cortisol in ethyl alcohol) in a round bottom flask using a rotary evaporator in a 45 C water bath.

---

<sup>a</sup>Mallinckrodt Chem., St. Louis, Mo.

Appendix Table 1 (Continued)

- 
- b. Resuspend the labelled corticoid with 1 ml ethyl alcohol.
  - c. Add 96 ml of triple distilled water.
  - d. Add 3 ml of dog plasma. (This plasma is collected from a dog adrenalectomized 36-48 hours prior to plasma harvest).
  - e. Mix the 100 ml solution by gentle swirling and store at 4 C at least one hour prior to use (average 224 pg cortisol/ml).
- 10. Mix the solution in the tubes by gently shaking the rack and place them in a 45 C water bath for five minutes.
  - 11. Place in 10 C water bath for 10 minutes.
  - 12. Add 15 mg Fuller's earth to each tube and shake the tubes in the rack for two minutes.
  - 13. Return rack of tubes to 10 C water bath for an additional 10 minutes.
  - 14. Centrifuge the tubes for two minutes at 2000 rpm.
  - 15. Place tubes in rack in 10 C water bath.
  - 16. Remove 0.5 ml of the supernatant fluid and place in a scintillation vial with an Eppendorf pipettor.<sup>b</sup>
  - 17. To each vial add 10 ml scintillation fluid using a repipet.
- 

<sup>b</sup>Brinkman Instruments, Westbury, N.Y.



## Appendix Table 1 (Continued)

---

The scintillation fluid was prepared as follows:

- a. Mix 5 g of diphenyloxazole (PPO) into 1 liter of toluene.
  - b. Add 100 ml Beckman Biosolv<sup>C</sup> #3.
  - c. Mix thoroughly.
18. Count each vial in a Beckman LS100 counter to a pre-set total count.
  19. Using the average time required to count each standard, plot a regression line and calculate its slope.
  20. Calculate corticoid content in each unknown sample, using a regression equation and the data obtained from the standards using the following equation

$$Y_i - \bar{y} = b(X_i - \bar{x})$$

The terms in this equation were defined as follows:

$Y_i$  = Time required to count the unknown.

$\bar{y}$  = Mean counting time for the standards.

$b$  = Slope of the regression line.

$X_i$  = Corticoid (ng/ml) in unknown.

$\bar{x}$  = Mean corticoid (ng/ml) of the standards

Solve the equation for  $X_i$  as follows:

$$X_i = \frac{Y_i - \bar{y} + b\bar{x}}{b}$$


---

<sup>C</sup>Beckman Instruments, Fullerton, Calif.

Appendix Table 2. Progesterone assay procedure

- 
1. Measure 2 ml of each plasma sample into a 16 x 125 mm screw top culture tube. Include three water blanks (2 ml triple distilled water) among the unknown samples and 2 progesterone standards (2 ml of 1 ng/ml and 5 ng/ml) at the end of each sample set.
  2. Add 10 nl of  $^{14}\text{C}$  labelled progesterone in methanol (activity approximately 170 cpm) to each tube in the sample set. Place 10 ml aliquots of the  $^{14}\text{C}$  labelled progesterone into three scintillation vials (one at the beginning, the middle and the end of each sample set). These vials are air dried and saved for use later in the test.
  3. Add 10 ml of redistilled or Nanograde petroleum ether<sup>a</sup> to each tube in the sample set. Shake each tube manually for 30 sec.
  4. Allow the tubes to stand for 5 minutes. Centrifuge the tubes for 15 minutes at 2000 rpm for separation of the plasma (water) and ether phases and freeze the plasma in an alcohol-dry ice bath.
  5. Pour off the ether extract into 15 ml conical centrifuge tubes while the plasma (water) remains frozen. Evaporate the ether with forced air and warming in a 45 C water bath.
- 

<sup>a</sup>Mallinckrodt Chem., St. Louis, Mo.

Appendix Table 2 (Continued)

- 
6. Repeat the extraction procedure (Steps 3, 4, and 5) two additional times.
  7. After the ether extracts are dry, rinse the sides of the conical tubes with 1 ml petroleum ether and evaporate to dryness. These tubes may be refrigerated until needed for spotting.
  8. Divide thin-layer silica gel chromatogram sheets (N-HR/UV 254, Machery-Nagel)<sup>b</sup> into 6 lanes. Spot progesterone standards in the outside lanes approximately 3 cm from the bottom edge.
  9. Spot the extract of each unknown on one of the inner layers, using 0.2, 0.1 and 0.1 ml of benzene-methylene chloride (1:1) sequentially to pick up and spot the extract.
  10. Develop the sheets in chromatography tanks containing diethylether-benzene (2:1) until the solvent front reaches near the top edge.
  11. Dry the sheets, locate the progesterone standards on the sheets with U.V. light and mark the samples accordingly.
  12. Scrap the samples with a razor blade broken to the appropriate size and mounted on a pencil. Place the silica
- 

<sup>b</sup>Purchased from Brinkman Instruments, Westbury, N.Y.

## Appendix Table 2 (Continued)

---

which peels off in curled bundles in Pasteur pipettes plugged with pyrex glass wool. The pipettes were prepared as follows:

a. The glass wool was washed in a sulfuric-nitric (2:1) acid bath and rinsed in distilled water.

b. The pipettes were plugged with the dry glass wool and rinsed with absolute methanol.

13. Elute each sample (silica gel in the pipette) with 3.0 ml absolute methanol into a 12 x 75 mm culture tube forcing the final portion through with rubber tube and glass mouthpiece.

14. Place 0.9 ml of the alcohol eluate in a scintillation vial which is air dried, filled with 10 ml of scintillation fluid and counted for  $^{14}\text{C}$  in a Beckman LS-100 counter. The scintillation fluid for  $^{14}\text{C}$  counting is prepared as follows:

a. 4 g diphenyloxazole (PPO)

b. 1 liter toluene, mix thoroughly.

15. Transfer two 0.9 ml aliquots of each eluate into two 12 x 75 mm test tubes and evaporate to dryness with forced air and a 45 C water bath.

16. Prepare triplicate standards of 0, 0.25, 0.5, 1.0, 3.0, 5.0 and 10.0 ng progesterone in 12 x 75 mm tubes. Dry as

Appendix Table 2 (Continued)

- 
- above and place one set of standards in each rack of samples (12 duplicate samples).
17. To each sample set and standard tube add 1 ml of 3% Corticoid binding globulin-<sup>3</sup>H-corticosterone. The CBG was prepared as follows:
- Measure 0.03 of <sup>3</sup>H-corticosterone into a round bottom flask.
  - Add 99 ml of triple distilled water.
  - Add 1 ml of dog plasma (adrenalectomized).
  - Mix and store at 4 C for at least one hour.
18. Shake the test tubes in the rack and place the rack in a 45 C water bath for five minutes. Transfer the racks to a 10 C water bath.
19. After 10 minutes, add 75 mg florisil to the tubes and shake slowly on a Vortex mixer for 30 seconds. Return the tube to the cold water bath for an additional five minutes.
20. Transfer a 0.5 ml aliquot of the supernatant with an Eppendorf pipettor into a scintillation counting vial.
21. Add 10 ml of scintillation fluid to each vial and count each vial for <sup>3</sup>H activity at 3% error in a Beckman LS-100 counter. The scintillation fluid was prepared as follows:
- 5 g of PPO
  - 1 liter toluene
  - 100 ml Beckman Biosolv #3.

## Appendix Table 2 (Continued)

- 
22. Plot the mean value (CPM) for each standard on semi-log paper and draw the standard curve.
23. Calculate the values for each sample using the following formula:

$$X_i = (ngV \cdot Cf) (Df - Bg)$$

Where  $X_i$  = sample value, ng progesterone/ml plasma

ngV = mean ng value of each sample (obtained from standard curve)

Cf = Correction factor for procedural loss

$\frac{\text{Average } ^{14}\text{C activity in 3 unprocessed vials-background}}{^{14}\text{C activity in each sample-background} \times 3.33 \text{ dilution}}$

Df = 3.33/2 (the dilution factor, since each sample was divided by 3.33 and 2 ml of sample was used)

Bg = Background (water blank values which are calculated before sample values are calculated)

---

Appendix Table 3. Estrogen assay procedure

- 
1. Pipette an aliquot of plasma (0.3 ml of utero-ovarian or 1 ml of Jugular plasma) into 16 x 125 mm screw cap tubes. Prepare two distilled water blanks for each assay.
  2. Extract the plasma samples with 10 ml anhydrous diethyl ether<sup>a</sup> (from a freshly opened can). Shake the samples for 30 seconds manually and allow 15 minutes before centrifuging for 10 minutes at 2000 rpm.
  3. Freeze the plasma (water) phase in a dry ice-alcohol bath and decant the ether extract into conical centrifuge tubes.
  4. Dry the ether extracts in a 45 C water bath under forced air flow.
  5. Add a second ether extraction (repeat steps 2, 3, and 4) to the conical tubes.
  6. Wash down each tube with 1 ml ether and dry again.
  7. Transfer the dried extracts with three rinses (0.3 ml of iso-octane (tri-methyl pentane) to prepared celite columns. The celite columns were prepared as follows:
    - a. Celite washed in GN HCL
    - b. Wash with triple distilled water until celite reaches pH 7.
    - c. Wash with methyl alcohol.
- 

<sup>a</sup>Spectrograde, Eastman Kodak Co., Rochester, N.Y.

Appendix Table 3 (Continued)

- 
- d. Wash with ether, dry and store in a muffle furnace at 540 C.
  - e. Mix celite with ethylene glycol 2:1 (W/V) and pack to a height of 5 cm in a 5 ml disposable pipette containing iso-octane.
  - f. Wash each column with 3 ml iso-octane before use.
  8. Separate the estrogens, by sequential elution of the columns under 2 psi air pressure until empty but not dry.
    - a. Wash with 8 ml iso-octane ----- discard
    - b. Wash with 4 ml 15% ethylacetate-iso-octane -----  
 $E_1$  fraction.
    - c. Wash with 5 ml 30% ethylacetate-iso-octane -----  
 $E_2$  fraction.
  9. Evaporate the separate tubes containing  $E_1$  and  $E_2$  fractions. Rinse with 1 ml ethyl acetate and evaporate completely.
  10. Prepare a series of standards as 0.2 ml aliquots from stock solution of estrogen in Tris HCl buffer,  
 $E_1$  = 0, 10, 25, 50, 100, 200, 300, 400, 500, and 600 pg estrone.  
 $E_2$  = 0, 5, 10, 20, 40, 100, and 200 pg estradiol.
  11. Add 0.6 ml tris-HCl-buffer to each dried eluate tube and mix with a Vortex mixer. The tris-HCl-buffer was prepared as follows:



Appendix Table 3 (Continued)

- 
- a. 1.21 g of THAM (tris hydroxymethyl aminomethane dissolved in 1 liter of distilled water.
  - b. Adjust pH to 8.0 with hydrochloric acid.
12. Prepare duplicate 0.2 ml aliquots of the Tris HCl-estrogen mixture in 12 x 75 mm tubes and add the prepared estrogen standards to the appropriate sample sets ( $E_1$  and  $E_2$ ).
  13. Add the following amount of reagents to the standards and sample sets:
 

	$E_1$	$E_2$
a. Tris HCl ethylene glycol buffer (80 ml Tris HCl buffer and 20 ml ethylene glycol.)	0.25 ml	0.24 ml
b. $^3\text{H}$ -estrone or $^3\text{H}$ -estradiol	0.03 ml	0.03 ml
c. Rabbit cytosol solution (see Appendix Table 4)	0.03 ml	0.04 ml
  14. Incubate the tubes over night (about 24 hours) at 4 C.
  15. Pipette 0.5 ml of charcoal-dextran-tris suspension to each tube and manually shake the tube rack. The charcoal-dextran-tris suspension was prepared as follows:
    - a. 1 g dextran
    - b. 1 g Norit A charcoal
    - c. 200 ml Tris-HCl Buffer
    - d. Mix and maintain suspension during use with a magnetic stirrer.
  16. Incubate the tubes for 15 minutes in a 15 C water bath.

## Appendix Table 3 (Continued)

- 
17. Centrifuge the tubes for 15 minutes at 2000 rpm and pour off the supernatant into counting vials.
  18. Add 10 ml of scintillation fluid to each vial and mix well. Scintillation fluid was prepared as follows:
    - a. 5 g of PPO (diphenyloxazole).
    - b. 1 liter toluene.
    - c. 200 ml Beckman Biosolv #3.
  19. Count the standards and sample sets for  $^3\text{H}$  activity in a Beckman LS-100 counter.
  20. Utilizing the counts per minute (cpm) for each standard set, compute the percentage of tritiated estrogen bound to cytosol for each standard, using the blank standard as 100% bound. Draw a standard curve of percentage bound for each standard set.
  21. Calculate the percentage bound for each sample in the sample set, using the water blank as 100%.
  22. Calculate the estrogen content of each sample utilizing the standard curve and the following formula:

$$Ec = \frac{Tt}{Ta} (Ev) \frac{1}{V}$$

where: Ec = estrogen concentration, pg/ml plasma.

Tt = 0.6 ml (total amount of tris-HCl used in each sample).

## Appendix Table 3 (Continued)

---

Ta = .2 ml (amount of tris HCl-estrogen used in each tube).

Ev = Estrogen value obtained for sample curve, based on % of  $^3\text{H}$  bound.

V = volume of plasma extracted.

---

Appendix Table 4. Preparation of rabbit cytosol solution

---

1. Uteri from six day pregnant rabbits are harvested, frozen on dry ice and stored at -20 C until needed.
  2. The uteri are homogenized in Tris-HCl Buffer (pH 8.0) with a Sorvall tissue grinder. The buffer containing 0.25 M sucrose and 0.001 M EDTA (ethylene-diaminetetra-acetate) should be used at a ratio of 1:3 (W/V) tissue to buffer.
  3. Centrifuge the homogenate at 3500 G for 15 minutes and save the supernatant.
  4. Recentrifuge the supernatant at 105,000 G for 10 minutes.
  5. Store the supernatant in small vials in liquid nitrogen until used.
-